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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 5 AUGUST, 1945 NUMBER 8

Fluorescence Studies of Carcinogens in Skin*

II. Mouse Skin after Single and Multiple Applications of 20-Methylcholanthrene

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(Received for publication March 16, 1945)

INTRODUCTION

The major cancer research project of this hospital is to ascertain how cancer develops in mouse epidermis under the influence of methylcholanthrene. During the past 7 years many properties of the changing epidermis have been measured quantitatively and others are now under investigation. Two summarizing reports of progress have been published (5). The principal purpose of these studies on fluorescence is to follow the course of the superficially applied carcinogen into the tissue and to discover how the methylcholanthrene and its fluorescent derivatives are related to the changes in epidermis leading up to cancer.

Recent fluorescence studies of tissues treated with chemical carcinogens have contributed enlightening information on (a) the manner of entry of these substances into the living organism (10, 13, 14, 16, 23); (b) the early structural and chemical changes in the tissues (3, 4, 6, 23, 25, 29); and (c) the metabolic alterations of the compounds themselves (1, 2, 11, 16, 17, 27, 28). Although chemical studies based on fluorescence methods for the identification and quantitative determination of carcinogenic hydrocarbons are numerous, only a few investigators have attempted to correlate the precise distribution of these compounds and their metabolites with the microscopic components of animal tissues.

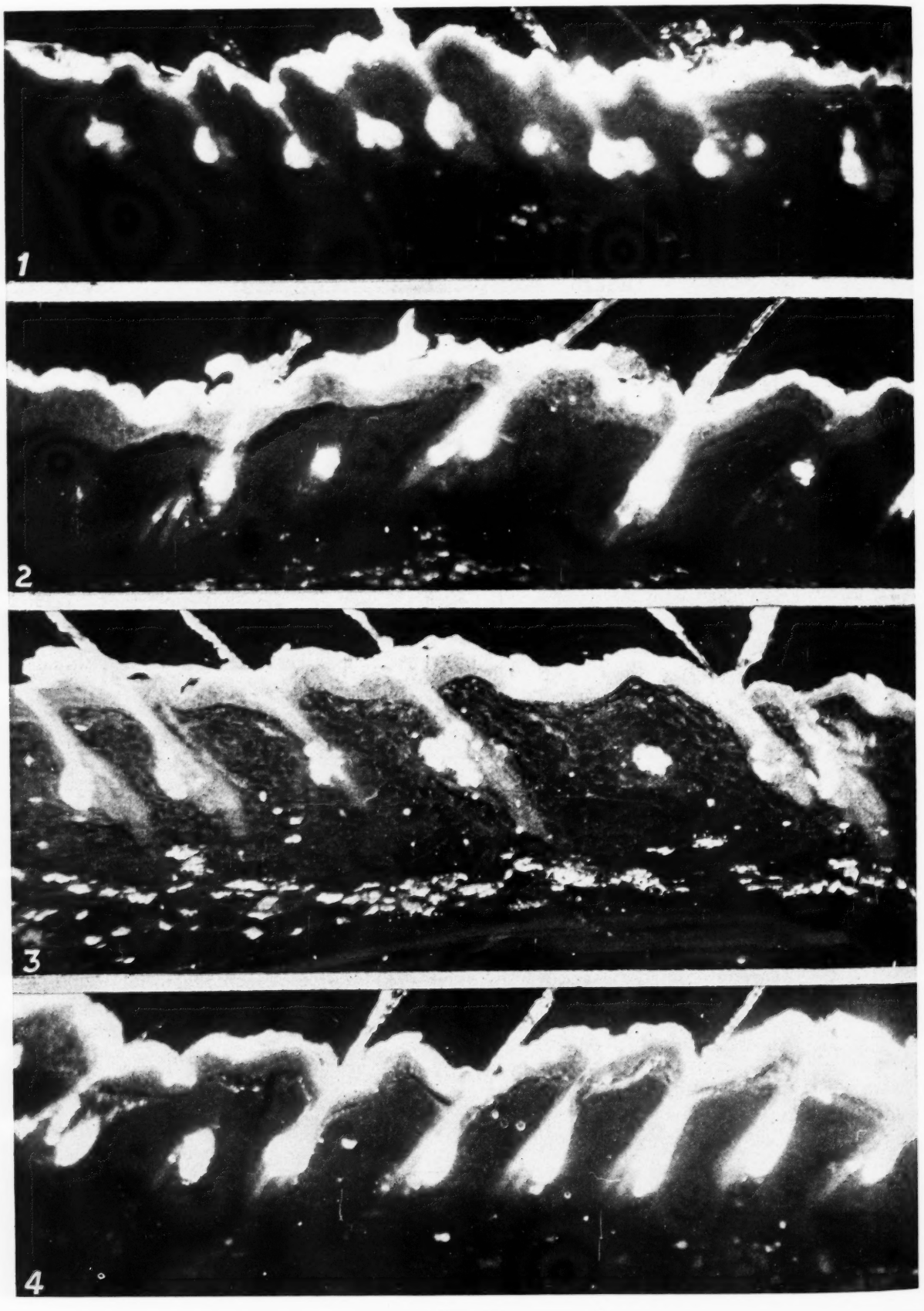
At the time Peacock (17) first reported the metabolic transformation of carcinogenic hydrocarbons after intravenous injection, he obtained from fluorescence microscopic examinations the direct evidence that liver cells took up the colloidally dispersed carcinogen, which then went into true solution within these cells. The changes in color and spectrum of the fluorescence of the hydrocarbon in the bile provided the first proof of the metabolic detoxification of the carcinogens. These observations were of much interest to those studying metabolism, but because the liver does not develop cancer in response to injected carcinogens they added nothing to the problem of distribution of carcinogens in susceptible tissues.

Graffi (13, 14) was apparently the first to use the fluorescence microscopic technic to study the accumulation of a chemically pure carcinogen in living mammalian cells. His observations on the storage of benzpyrene were limited to cells in tissue cultures, and provided no answer to the question how the carcinogens are taken up by the tissues of intact animals.

The localization and persistence of cutaneously applied carcinogens in the intact organism have recently been studied independently in two laboratories. Doniach, Mottram, and Weigert (10, 11) have determined, chiefly by fluorescence spectrographic methods, the persistence of 3,4-benzpyrene and its fluorescent decomposition products in skin and other tissues after a variety of methods of application, and have attempted to establish the relationship of the fluorescent breakdown products to each other and to the parent hydrocarbon. We (23) have previously reported on the histological localization of methylcholanthrene after a single painted application to mouse skin.

In so far as these two investigations overlap there appears to be great similarity in the distribution, persistence, and excretion of the two carcinogens employed. Doniach, Mottram, and Weigert did not examine sections of tissues and were able to interpret the question of histological localization of fluo-

* Aided by a grant from an anonymous donor.



FIGS. 1-4

rescence only from the appearance of cleared whole mounts of skin. On the other hand, in our observations, made on frozen sections, we were concerned chiefly with the selective localization of the unchanged carcinogen in the skin, and considered the weak blue fluorescence of the primary decomposition product of the carcinogen to be an artefact. The appearance and distribution of the blue-fluorescent substance has been reinvestigated and will be described in a subsequent section of this report.

As has been reported in previous papers from this laboratory, a single cutaneous application of methylcholanthrene in benzene will produce carcinomas in from 20 to 40 per cent of mice of the New Buffalo and Swiss strains (7, 22). The distribution of methylcholanthrene in the skin of such mice after a single application, and its subsequent disappearance from there, was described in the first paper of this series (23). Since repeated applications of this same chemical will initiate carcinogenesis in the 60 to 80 per cent of mice that are refractory to a single treatment (8), it would appear that some sort of cumulative structural and functional alteration of the skin occurs in response to successive applications. We now describe the histological distribution of methylcholanthrene when applied to mouse skin that has been altered by previous treatments with the same agent. In the course of these observations other and new fluorescent substances, which had developed in the tissue, were noted. A preliminary description of these is included.

MATERIAL AND METHODS

Two general groups of animals were used in this study. The first included approximately 80 young Swiss mice of both sexes. These were painted at 14 day intervals with one brush stroke each of a benzene solution containing 0.6 per cent methylcholanthrene. Mice from this group were killed at intervals of from a few minutes to 10 days after the second, third, or fourth treatment, and at intervals up to 6 weeks after the fifth and last painting.

The second group included more than 100 mice bearing skin tumors induced by methylcholanthrene applied in various ways. Both the cancerous and the noncancerous skin areas of the animals were studied. Noncancerous skin areas showed hyperplastic changes varying in degree and in extent.

Frozen sections for fluorescence studies were usually cut 20 microns thick, after fixation of the skin from 12 to 24 hours in 10 per cent reagent grade formalin, but some preparations were cut without prior chemical fixation to study the effect of formalin on the fluorescences observed.

A comparison was made in some cases, also, with the fluorescence that could be seen in paraffin sections cleared in benzol and mounted unstained in nonfluorescent mineral oil or in Clarite X. Lipid distribution was studied in the frozen sections by staining with Sudan IV for bright field microscopy and with Phosphin 3R for the fluorescence microscope. Frozen sections stained with toluidine blue, thionin, and methylene blue were used for orientation and for the study of the distribution of certain connective tissue cells.

OBSERVATIONS

Appearance of a blue-fluorescent substance in skin after a single application of methylcholanthrene in benzene.—In an earlier paper of this series (23) the distribution and subsequent disappearance of methylcholanthrene after a single cutaneous application to the dorsal skin of mice was described. While this process was under examination it was recognized that the fluorescence of the deeper layers of epithelium was enhanced beyond that of normal epidermis within a day or two after the painting (Fig. 2). Because the intensity of this blue fluorescence apparently increased and spread to other structures, including dermal connective tissue fibers and even muscle, after sections had lain about the laboratory for some days, it was first interpreted as an artefact of decomposition (Fig. 3). The reports by Doniach, Mottram, and Weigert (10, 11, 16) on the presence in mouse skin of a blue-fluorescent, alkali-soluble derivative of 3,4-benzpyrene led us to reinvestigate the source of the fluorescence, which we also had recognized. Their

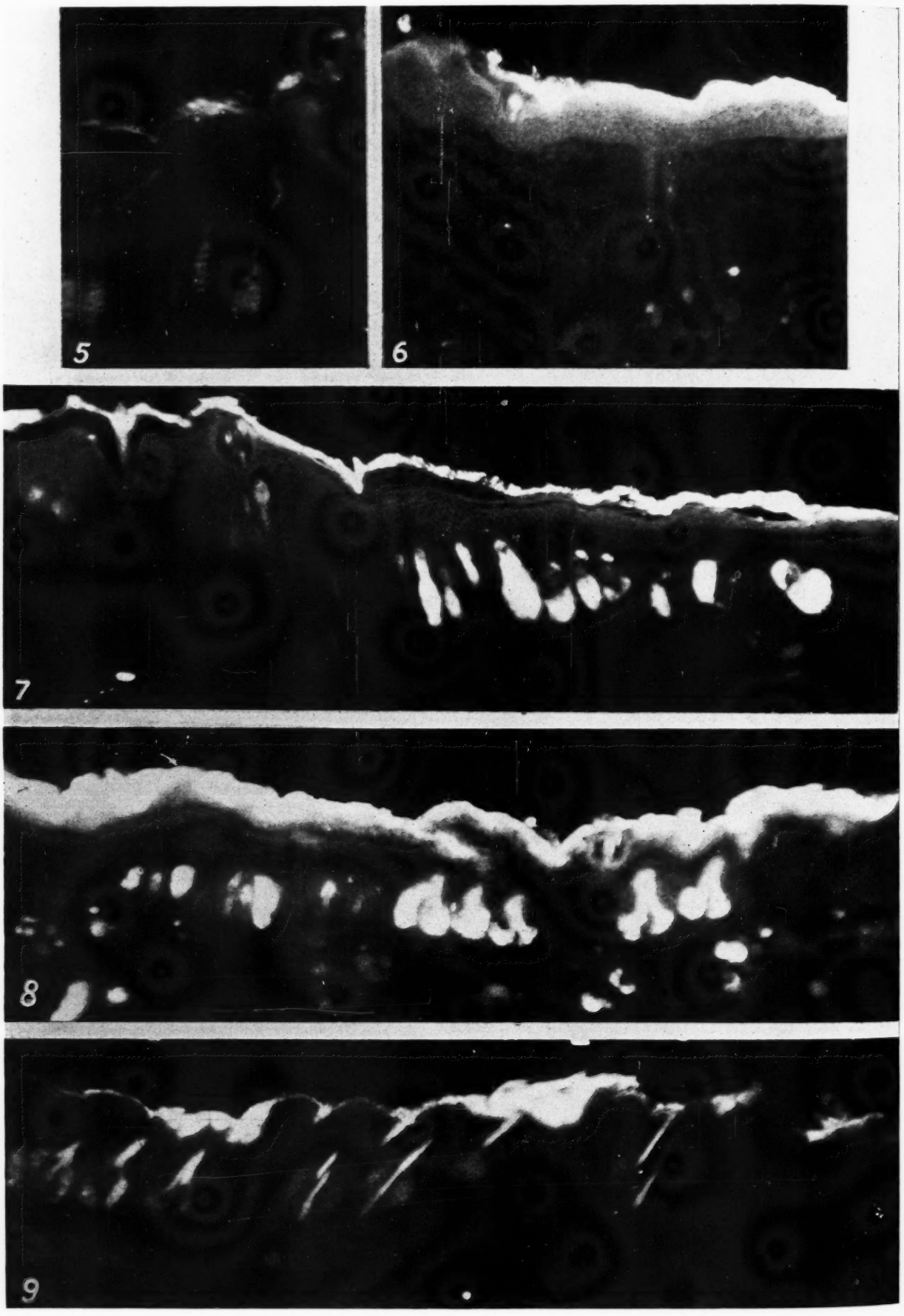
DESCRIPTION OF FIGURES 1 TO 4

FIG. 1.—Fluorescence of methylcholanthrene in axial frozen section of formol-fixed skin of Swiss mouse, killed a few minutes after first application of benzene solution of carcinogen. Mag. × 135.

FIG. 2.—Mouse skin 2 days after first application of methylcholanthrene. Intense blue-violet fluorescence confined to surface, remnants of sebaceous glands, and cells of subcutaneous fatty layer. Weaker blue fluorescence of "MCX" appears in cytoplasm of deeper epidermal cells but not in their nuclei, which appear as dark spots in photograph. Mag. × 135.

FIG. 3.—Enhancement of fluorescence in section photographed one week after it had been prepared. Mouse killed 2 days after first painting. Both epidermal and dermal structures show much more fluorescence than in freshly prepared section such as used for Fig. 2. Even here, nuclei show no accumulation of fluorescent substances. Mag. × 135.

FIG. 4.—Toxic effect of methylcholanthrene on deeper epidermal cells. Another area from same skin as used for Fig. 2. Here intense methylcholanthrene fluorescence occurs in droplets of free lipid, readily demonstrable by Sudan IV staining in deep epidermal cells.



FIGS. 5-9

report was based chiefly on spectrographic data obtained from whole skin although they also examined, at very low magnifications, cleared whole mounts of skin. They concluded that the blue fluorescence was localized chiefly in the hair bulbs. In our fluorescence microscopic specimens it was evident that the strongest blue fluorescence appeared in the uncornified layers of surface epithelium, and that less was visible in the epithelium of the hair follicles. Viewed from the surface at low power, the greater thickness of epithelium at the hair follicles might easily make it appear that the fluorescence was most intense in such areas. Our studies also showed certain finer details of the distribution of this blue-fluorescent material. In all cases it was confined to the cytoplasm of the epithelial cells. It therefore either failed to penetrate into the nucleus or was quenched at once if it did penetrate (Fig. 2).

Further studies were then undertaken to determine whether this fluorescence was present in freshly prepared frozen sections of once-painted skin cut without chemical fixation, or was an artefact of fixation and storage of the skin. Frozen sections of unfixed skin showed the blue fluorescence just as did those of formol-fixed specimens. Immersion of the fresh sections for 24 hours in a 10 per cent aqueous formol solution produced no change in the distribution or intensity of this fluorescence. It must be concluded that the blue fluorescence is not an artefact, although it does become much more intense in old sections (Fig. 3). Examination of a series of skins from mice killed at intervals after a single painting revealed that the fluorescence appeared within a day after application of the carcinogen, and that it was detectable for at least 2 to 3 weeks. After the removal of unchanged methylcholanthrene by extraction with alcohol and benzene, the blue fluorescence appeared unaffected in intensity. We have not attempted to prove by chemical methods that the blue-fluorescent compound is actually derived from methylcholanthrene, but it probably is. It resembles in its solubility properties the blue-fluorescent benzpyrene derivative investigated by Doniach, Mottram, and Weigert in mouse skin, in that it is removed by extraction with 0.01 *N* NaOH and passes into an ether layer after acidification of the extract with HCl.

These findings suggest that methylcholanthrene is metabolized in the tissue in the same manner as is 3,4-benzpyrene. The blue-fluorescent substance in methylcholanthrene-painted skin differs from that studied by Doniach, Mottram, and Weigert in that it undergoes fairly rapid photodecomposition in near-ultraviolet light. The intensity of the fluorescence fades and within a few minutes completely disappears when exposed to intense near-ultraviolet radiation on the stage of the fluorescence microscope. More precise studies on the photochemistry of methylcholanthrene and its breakdown products had been initiated in cooperation with Drs. Knorr and Albers, of the Kettering Foundation for the Study of Chlorophyll and Photosynthesis, at Antioch College, but had to be interrupted because of the war.

Distribution of Methylcholanthrene in Mouse Skin after Repeated Applications

Striking structural and chemical changes are produced in mouse skin within a 2 week period following a single application of methylcholanthrene in benzene. Some of these effects have been summarized by Cowdry (5) in a recent review of the work in this laboratory. Although these chemical changes, averaged by the fact that they are based on pooled specimens from 10 mice, are in good agreement, the histological pictures of individual skins are extremely varied 2 weeks after one painting. The epidermis of such treated skin may show a general hyperplastic response, or it may be thin and damaged. Often a complete range of effects may be observed in a single mouse. A central longitudinal strip may be thinly covered by epidermis and be paralleled on each side by a ridge of greatly thickened hyperplastic epidermis, in which the cells are large and contain hypertrophied nuclei rich in deeply stained chromatin. Sebaceous glands are destroyed by the initial carcinogen treatment and usually do not regenerate by 2 weeks. Centrally the hair follicles are usually absent or at least reduced to short thick epithelial pegs, which often contain cystic, keratin-filled cores. These general morphological features of the once-treated skin influence the entry and persistence of carcinogens subsequently applied. Less obvious differences between skins, such as content of free lipids and changes in cell permeabilities, may also influence the response to further treatments.

All mice killed within a few minutes after the last of multiple applications of methylcholanthrene showed yellow-green fluorescent crystals of the car-

DESCRIPTION OF FIGURES 5 TO 9

Fig. 5.—Methycholanthrene fluorescence in large sebaceous glands that remain occasionally in epilated areas. Duct of one gland seen twisting toward epidermal surface. Mouse killed 10 minutes after second application of methylcholanthrene. Mag. × 135.

Figs. 6 to 9.—All are from skin of one mouse killed a few minutes after third application of methylcholanthrene. See text for description. Mag. × 135 in all figures.

cinogen on the surface of the skin, as well as a strong blue-violet fluorescence of the dissolved carcinogen within the superficial keratin layer whenever such a layer was present. Within the outermost cells of the subcutaneous fatty layer similar blue-violet fluorescence

destructive action of methylcholanthrene, and when present take up the carcinogen and fluoresce brightly blue-violet (Fig. 5). They are often deep in the dermis, in areas containing few or no hair follicles, and may open directly on the surface by long, tortuous ducts.

FIG. 10
(See bottom of page 455 for description)

may be present to a variable degree, depending on the thickness and free lipid content of the overlying layer of epidermis and on the amount of carcinogen applied. Although sebaceous glands are not usually present in such painted areas, hypertrophied glands do occasionally exist at the edges of the previously treated skin area. These glands tend to occur in a patchily irregular distribution, especially after several applications. Such regenerated glands are apparently resistant to the

The ducts occasionally contain enough methylcholanthrene to be outlined distinctly by its fluorescence.

Not infrequently the whole thickened portion of the epidermis shows a moderately strong blue-violet fluorescence immediately after repeated painting. This contrasts sharply with the distribution of the carcinogen in a previously untreated epidermis, which has never been seen to take up directly fluorescent methylcholanthrene in the uncornified deeper layers. The

fluorescent material in such repainted epidermis is diffusely distributed, and appears to be in every part of the cytoplasm. The nuclei appear dark against this fluorescent background (Figs. 6, 7, 10, 11). Fluorescence spectrographic data indicate that this fluorescence is due to unaltered methylcholanthrene. The significance of this direct absorption of methylcholanthrene by viable epithelial cells will be discussed later.

An illustration covering the usual range of the variations to be found in different areas of one and the same skin after several applications of a benzene solution of methylcholanthrene is shown in Figs. 6 to 11, inclusive. All are from representative areas of sections cut across the skin perpendicular to the axial direction of the brush strokes made when the carcinogen was applied. The mouse was sacrificed a few minutes after the third painting. The central area of strong but fairly regular hyperplastic reaction is shown in Fig. 6. Blue-violet fluorescence of methylcholanthrene is to be seen throughout the thickness of the epidermis, very intense in the lipids of the superficial keratinized portion, and moderately strong in the cytoplasm of the cells of the deeper portion. Only a few pegs project into the dermis as remnants of the former hair follicles; the blue-violet fluorescence in these pegs fades off as the distance from the surface increases. Deep, keratin-filled, cystic remnants of hair follicles are so weakly fluorescent as to be recognized only with difficulty in the photograph. Only a little methylcholanthrene reaches the most superficial fat cells beneath this epithelium.

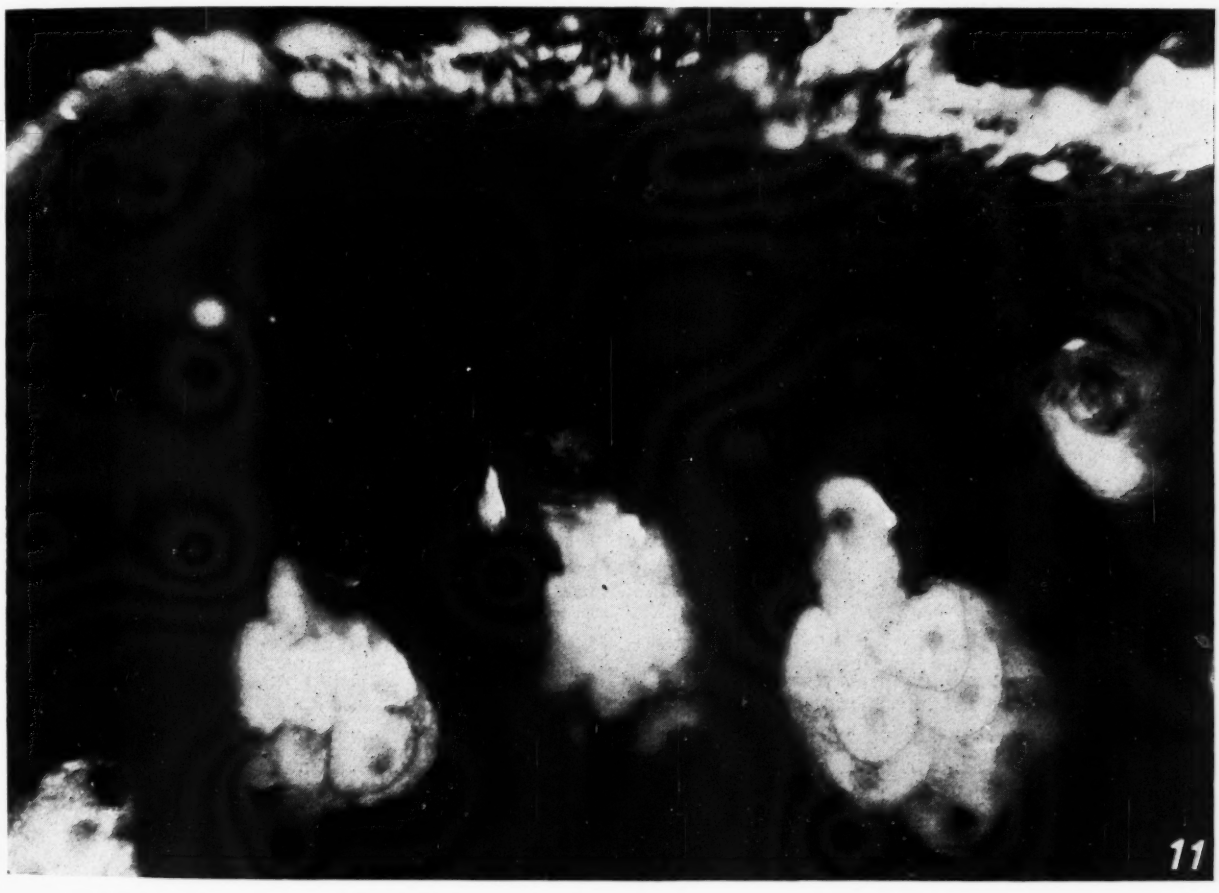


FIG. 11

DESCRIPTION OF FIGURES 10 AND 11

FIGS. 10 and 11.—Oil immersion photographs of areas from same sections as used for Figs. 6 to 9. Both are at edges of strongly hyperplastic epidermis in region where large sebaceous glands persist. Strikingly brilliant fluorescence of methylcholanthrene occurs in droplets of varying size in sebaceous glands. Central dark spots in cells obviously represent nuclei. Especially at lower edge of Fig. 11 nuclei are seen without much cytoplasmic covering, and there appear completely black. Weaker fluorescence of dissolved methylcholanthrene also seen in cytoplasm but not nuclei of entire epidermal layer. Close inspection reveals that this appears to be finely granular, as if it were adsorbed on, or dissolved in, cytoplasmic granules. In center of Fig. 11, just above central sebaceous gland, fluorescence of methylcholanthrene seen in epithelium of a hair follicle, but here is much weaker than that of surface epithelium.

At the sides of the central hyperplastic strip the thickness of the epidermis decreases (Fig. 7), and soon approaches that of the normal untreated skin (Fig. 8). In both of these areas, however, the previous treatment has so modified the deeper epithelial cells that they freely take up methylcholanthrene and emit its distinct blue-violet fluorescence. Such areas vary considerably in the amount of lipid present in the keratinized surface, and therefore differ greatly also in the amount of blue-violet fluorescence in the keratin layer. This contrast is apparent by comparison of Figs. 7 and 8. In the photographs no clear differences indicate what part of the superficial light layer is due to blue-violet fluorescence of dissolved methylcholanthrene and what represents yellow-green fluorescence of the crystallized excess carcinogen. Actually there was almost no superficial blue-violet fluorescent layer in the area of Fig. 7, and so the light layer indicates only yellow-green fluorescence. In general this characterizes the differences observed in the amount of superficial lipid; the keratin layers of repainted hyperplastic areas are quantitatively poor as a rule in free lipids, although, as mentioned earlier, considerable variability may exist within the same skin.

As is often the case, patches of skin containing sebaceous glands are noted at the edge of the hyperplastic area (Fig. 7), and occur more or less regularly in the less affected regions that have shown minimal reactions to the previous painting (Fig. 8). These glands are usually quite irregular in shape, and frequently are associated only with an epithelial peg or isolated cellular nest instead of a typical hair follicle. Often no evidence of the old hair follicle remains, and the glands either open directly on the epidermal surface or empty into a closed epithelial sack.

Figs. 10 and 11 show areas of moderate hyperplasia and the methylcholanthrene-filled sebaceous glands beneath at higher magnifications. Dark round and oval spots, optically empty, are noted where nuclei are present in the epidermal cells and in the cells of the sebaceous glands. No evidence of characteristic fluorescence has been recorded by us to suggest that methylcholanthrene enters the nucleus of living cells. From these figures it is clear that methylcholanthrene fluorescence is distributed in sebaceous cell cytoplasm in the same way as the lipid droplets. At the neck of the gland the fluorescence is diffuse, corresponding to the zone where lipid droplets coalesce to become the definitive secretion, or sebum.

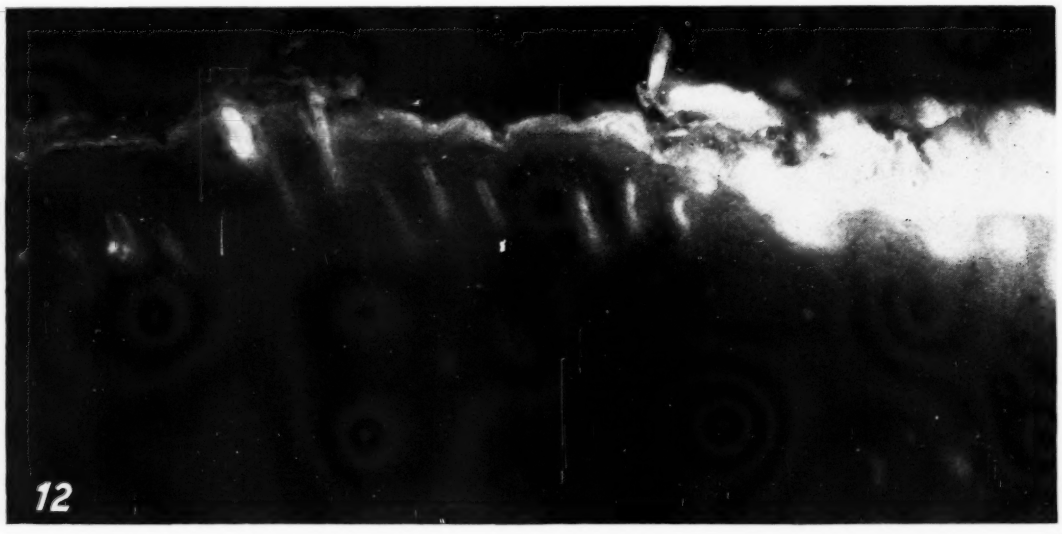


FIG. 12.—Mouse skin 3 days after second application of methylcholanthrene in benzene. Central area, at left, covered with very thin epidermis from which methylcholanthrene has disappeared. At right, the thick keratin contains much methylcholanthrene in solution in free lipids. Beneath this a considerable amount of blue-fluorescent 'MCX' seen in hyperplastic epidermis.

The cytoplasm of deeper epidermal cells (Figs. 10 and 11) also shows blue-violet fluorescence, but of much less intensity than in the sebaceous glands. The intensity is too low to permit certain determination of the distribution of the fluorescence by direct visual observation. In photographs, made with a reduced objective aperture that improves the resolution, the fluorescence appears to be slightly mottled, which may be interpreted as evidence that the carcinogen is adsorbed onto some preformed cytoplasmic constituent. Experiments are being undertaken to answer this question by more direct methods.

Fig. 9 has been included to facilitate a direct comparison of nonhyperplastic, previously painted skin (Fig. 8), and normal skin of the same animal just outside the area to which methylcholanthrene had been

applied earlier (Fig. 9). The last application, made just before the mouse was killed, extended well to the sides of the earlier paintings. In Fig. 9, although a strong fluorescence is present on the surface and in the sebaceous glands, there is no fluorescence of the deep epidermis to suggest that any carcinogen has entered these cells. This corresponds to the absorption noted after a single application (Fig. 1) and contrasts sharply with the absorption in the adjacent previously painted skin (Fig. 8).

Loss of the yellow-green surface fluorescence of crystallized excess methylcholanthrene from repainted skin is usually very rapid. Most mice examined in ultraviolet light 2 days after the last painting showed no macroscopic signs of the dry carcinogen.

Since great variations exist in the previously treated skins and in the manner in which these skins absorb newly applied methylcholanthrene, it is not surprising that great differences exist in the manner of disposal of the carcinogen. It has been indicated that only small amounts of evident methylcholanthrene penetrate areas of thin epidermis that are extensively damaged and even ulcerated. Confirming the macroscopic observations, one finds microscopically that crystals of dry carcinogen are infrequent after a day or two on the surface of such areas (Fig. 12). Likewise little or no blue-violet fluorescence attributable to unchanged dissolved methylcholanthrene is found in the epithelium itself, or in the dermis except for occasional traces in the superficial portion of the fatty layer. Since neither hair follicles nor sebaceous glands usually remain in such an area there is no direct path that the carcinogen may follow into the deeper parts of the dermis. Loss of fluorescence from the fat cells occurs, as it does after a single painting, by a slow diffusion into the deeper fatty layer, from which it fades slowly. After 5 or 6 days the fat cells show only their normal dull fluorescence.

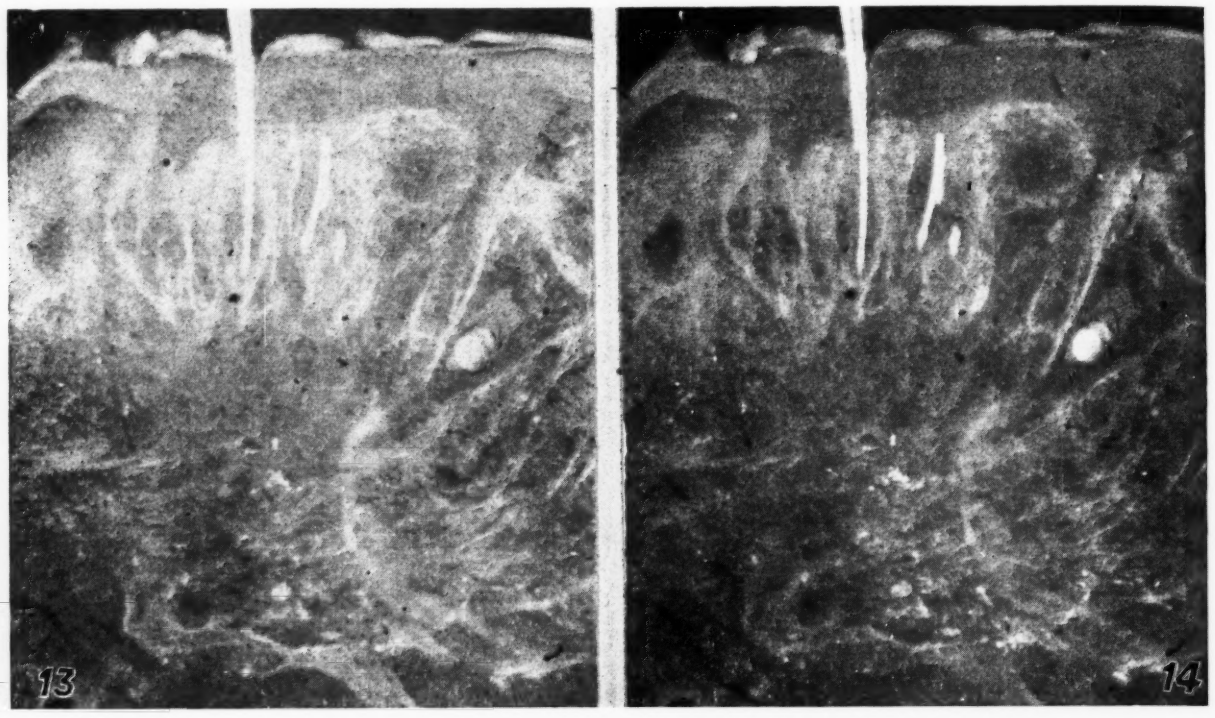
The disappearance of methylcholanthrene from thick hyperplastic epidermis occurs differently. Sections through such areas continue to show blue-violet fluorescence for several days in the free lipids of the hyperkeratinized crust. Infrequently traces of methylcholanthrene fluorescence have been observed in such keratin several weeks after the last painting. It is improbable that this carcinogen can have any effect on the deeper viable tissues, however, since it remains isolated and is finally shed with the keratin as desquamation occurs.

The methylcholanthrene that permeates the cytoplasm of viable hyperplastic epidermal cells is disposed of otherwise. The blue-violet fluorescence encountered in such repainted epidermis has been shown by its fluorescence spectrum to be due to unaltered methylcholanthrene. This fluorescence persists for 2 or 3 days without appreciable alteration other than a slight fading. By the third or fourth day, the fluorescence present in the epithelium is altered to appear less violet and more distinctly blue in color. This change proceeds until, at 6 or 7 days after painting, the color is unequivocally blue, indicating the absence of unaltered methylcholanthrene. This blue fluorescence may persist for several weeks following the last painting. It is probably due to the same compound that produces the blue fluorescence that penetrates the epithelium after a single initial application of the carcinogen, since its response to alkali extraction and to other rough qualitative tests is identical. The actual time of persistence of this substance is difficult to determine by the fluorescence microscopic technic alone, for, as will be described in detail later, there are blue fluorescences encountered long after painting that are dependent on the formol fixation of the tissue. Since the periods at which these blue fluorescences occur are contiguous, and may even overlap, the identification of either must rest on more than microscopic observation of the fluorescence in fixed sections.

Fluorescence of skin in later stages of methylcholanthrene carcinogenesis.—Skins of mice examined for fluorescence some months after the last application of methylcholanthrene were limited largely to those bearing one or more malignant tumors on some part of the skin of the back. The nontumorous parts of these skins ranged from those that were macroscopically normal to those that were epilated, hyperplastic, or frankly precancerous on histological examination. Sections were usually cut to include both the tumor and the noncancerous skin.

Specimens of this sort present a fluorescence microscopic picture that is vastly different from normal skin, as well as from the skin of early stages in methylcholanthrene hyperplasia. Dermal structures that can be readily recognized by their fluorescences include the keratin, which fluoresces light bluish-white or sometimes greenish-white, and certain accumulations of cells and fibers in the dermis. A histological account of the response by tissue mast cells in the dermis beneath hyperplastic epidermis has appeared as a separate paper (6). The studies of other aspects of the dermal reaction and of the striking golden-brown fluorescence of certain of the mast cells will be published later. At present only the fluorescences observed in epithelial structures will be considered.

In contrast to the dark blue fluorescence that arises from deeper layers of normal mouse epidermis under ultraviolet illumination, areas of hyperplastic epidermis and parts of papillomas and carcinomas fluoresce strongly light blue after formol fixation



FIGS. 13 and 14.—Mouse killed 6 months after last of 5 applications of carcinogen, when a fully developed cancer was present. Area includes hyperplastic epidermis adjacent to carcinoma. Blue fluorescence uniform throughout epithelial structures, and of type found only after fixation of tissue. Loss of fluorescence with irradiation is illustrated. Figures are from identically exposed films, but Fig. 13 was taken as soon as area was placed in field while Fig. 14 was made after 30 minutes' irradiation. Mag. × 100 (approx.).

(Fig. 13). In benign and malignant epithelial neoplasms this fluorescence occurs especially in the well differentiated areas that seem, histologically, to be on the way toward keratinization.

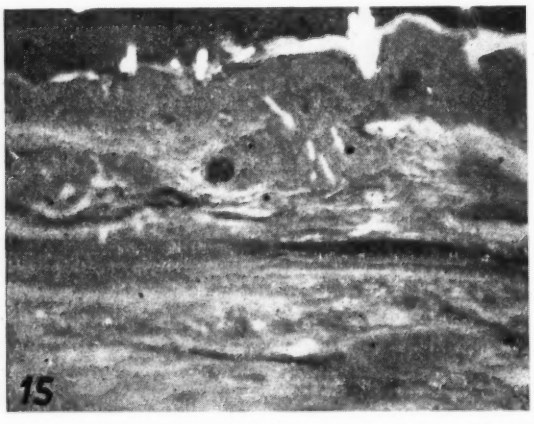


FIG. 15.—Fluorescence of slightly hyperplastic epidermis of mouse killed 6 months after last application of methylcholanthrene. Epidermis fluoresces bluish and appears brighter than adjacent dermis, a reversal of condition in normal untreated skin. Mag. × 100 (approx.).

Less well differentiated carcinomatous epithelium varies greatly in its fluorescence, but usually appears in colors ranging from deep reddish purple to very dark blue, or even blue-violet. The reddish tinge in freshly prepared sections is usually associated only with large epithelial masses that are adjacent to necrotic areas, and are probably themselves undergoing early stages of necrosis. We have not attempted to determine the source of this reddish fluorescence, but in the light of observations by Policard (18) and Körbler (15) one might expect it to be due to porphyrins liberated from blood in such devitalized areas. Figge (12) has recently reviewed the possible relationship of porphyrin metabolism to cancer, especially as these agents may act as sensitizing agents to ultraviolet light. He has suggested, also, that a relation probably exists between porphyrins and susceptibility to other carcinogenic agents, although the mechanism of such a relationship was not clear. In our studies fluorescences probably attributable to free porphyrins were encountered only in association with areas of decomposition, and rarely in the superficial, keratinized layer of the epidermis (Fig. 16). Red fluorescence does occur at the latter site rather regularly in old sections that have been kept some days after being mounted in glycerine.

The blue-fluorescent material of these sections is not the same as that observed within a short time after methylcholanthrene painting. It is not removed by alkali extraction of the sections, and does not appear in the skin until the specimen has been fixed in formol. Frozen sections of such skin cut without prior fixa-

tion contained very little fluorescent material in the epidermis, but a characteristic blue fluorescence developed slowly in the epithelium of these sections as they were allowed to soak in a 10 per cent aqueous solution of reagent grade formalin. The material appears most strongly fluorescent in the areas of greatest hyperplasia, although areas of thin epidermis at the sides (*i.e.*, outside the central painted area) also show a definitely stronger fluorescence than does normal epidermis (Fig. 15). This blue fluorescence fades slowly on exposure to ultraviolet light, indicating that photodecomposition has occurred. This can be appreciated by examination of Figs. 13 and 14, which show the same area of hyperplastic skin. The first photograph was taken as soon as the section was placed on the microscope; the second, an identical exposure, after 30 minutes' irradiation. There is little apparent change in the dermal constituents, while the fluorescence recorded from epithelium is significantly weaker. Evidence has not been obtained on the specific nature of the substances responsible for this late appearing blue fluorescence. The properties investigated suggest that it is different from the substance that gives rise shortly after the application of methylcholanthrene to blue fluorescence. The visual similarity of the two blue fluorescences may result in some confusion in the interpretation of the roles played by various metabolic derivatives of the carcinogen, unless chemical or spectrographic methods are employed to determine which blue-fluorescent material is actually present.

The keratin that piles up on a precancerous epidermis and the nests of keratin that appear in the dermis, as well as in the stroma of malignant tumors, fluoresce brilliantly white with a blue or greenish tinge. This fluorescence is not affected by fixation, and is easily recognized even on superficial clinical examination of hyperkeratinized precancerous lesions of man in filtered ultraviolet light (19, 20).

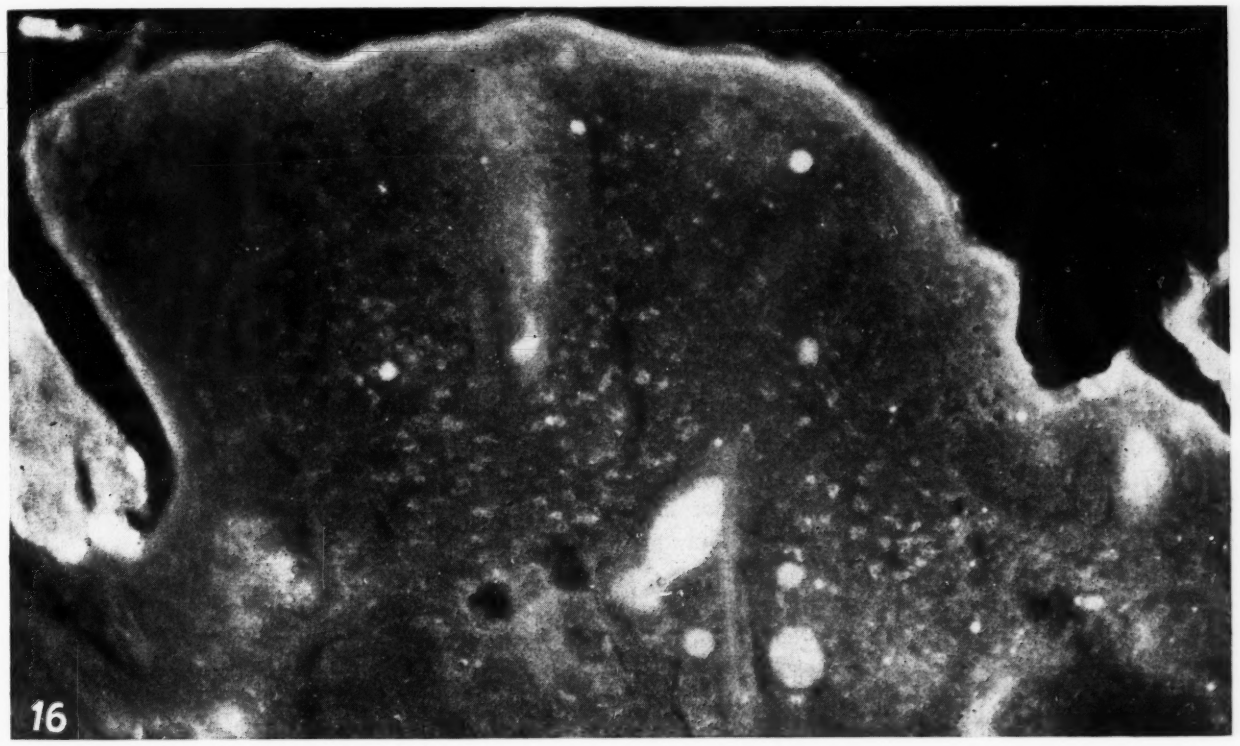


FIG. 16.—Small papillary growth on skin of mouse killed 2 days after fourth painting with methylcholanthrene. All trace of typical methylcholanthrene fluorescence was absent from this area, and instead the bright surface streak represents bright salmon pink fluorescence. Such fluorescence occurs rarely at this site in fresh tissue. Mag. × 135.

DISCUSSION

After the first pure carcinogenic hydrocarbons were made available, it soon became apparent that one of the most interesting and most promising directions for cancer research was the biochemical study of the distribution and persistence of these compounds in the animal body. At first crude, and lately precise, data have been recorded on the nature of the metabolic alterations of the carcinogens in animal tissues. But little has been done toward the correlation of these significant findings with the mass of information that has accumulated on the rapid and profound structural changes that occur in carcinogen-treated tissues. Aside from Graffi's observations on carcinogens in tissue cultures, only our previous report has presented

data on the precise localization of carcinogenic hydrocarbons when they are first applied to mammalian tissues, and on how long they persist in an unchanged condition at these sites. Our observations have now been extended to the distribution of methylcholanthrene and its fluorescent derivatives throughout the period of chemical carcinogenesis.

As a result of this study, it can be shown that one early effect of the carcinogen is to alter the permeability of living epithelial cells to subsequently applied methylcholanthrene. This finding supports further a considerable body of evidence that has been previously reported on the rapid and striking changes induced in the epidermal epithelium by a single treatment with methylcholanthrene in benzene solution. Changes occurring within a few days as a result of such a single painted application include the increase in volume of chromosomes (3), a massive decrease in calcium and iron content (4, 25), and a reduction in the total amount of lipids (29).

When first applied in benzene, methylcholanthrene does not penetrate the living cells of the deeper layers of the epidermis in an unaltered state. If, several days after the first application, the unchanged carcinogen is found in the epidermal cells, it is always present in intracellular droplets of free lipid in cells that show early evidences of degeneration (Fig. 4). Not infrequently, however, a part of the second application of the same carcinogen passes at once into deeper cells of the now thickened epidermis. Spectrographic data show that this fluorescence arises from unaltered dissolved carcinogen. In these skins the fluorescence suggests that the carcinogen is diffusely spread throughout the cytoplasm, or possibly adsorbed to some cytoplasmic granules, and is absent from the nuclei of cells that show no evidence of degenerative changes. The preceding treatment of the skin with methylcholanthrene appears to have so modified the epithelial cells that they are now able to absorb a substance to which, in their normal condition, they are impervious. The nuclear membrane seems to be impermeable, even after multiple treatments of the skin with the carcinogen.

These data offer further proof of the essential difference between normal epithelial cells and the cells that appear in the epidermis after one or more treatments of the skin with methylcholanthrene. Normally, a physiological barrier protects the living epithelial cells against entry of the unaltered carcinogen. If that barrier be broken down, and possibly it is broken down only after the entry of the blue-fluorescent decomposition products of the carcinogen, the methylcholanthrene enters the cell, to go into solution in droplets of lipid that are probably the result of cytoplasmic disintegration. Such cells are almost certainly destroyed and shed. In their place cells regenerate with an increased resistance to the toxic effects of the carcinogen. It is probable, also, that cells that have once taken up the blue-fluorescent derivative, but not unaltered methylcholanthrene, share the modified reaction to subsequently applied carcinogen. Such cells, whether physiologically altered original cells or cells that have regenerated, are permeable to the carcinogen but appear to be resistant to its destructive action. They take it up diffusely in their cytoplasm and transform it, *in situ*, to the blue-fluorescent, alkali-soluble derivative.

It is of some interest to consider the results of this investigation in the light of spectrographic and chemical studies by Doniach, Mottram, and Weigert (10, 11) and by Berenblum and his collaborators. The former workers have shown that, shortly after 3,4-benzpyrene is applied to mouse skin, a blue-fluorescent compound appears in the skin and persists for several weeks, the carcinogen itself being absent after about a week except for that remaining on the fur. From their study of cleared whole mounts of skin at very low magnifications they believed that this compound was localized chiefly in the hair bulbs. From spectrographic evidence they concluded that this blue-fluorescent compound was not identical with the metabolic derivative of benzpyrene that Peacock (17) first described and that has recently been determined by Berenblum (1) to be 8-hydroxy-3,4-benzpyrene.

A blue-fluorescent substance also appears in the epithelium after an initial application of 20-methylcholanthrene. The fluorescence microscopic technic makes it possible to localize this substance in the cytoplasm of the living cells of the epithelium, especially those of the epidermis. The behavior of this compound towards solvents parallels the behavior of the alkali-soluble substance derived from benzpyrene.

It seems that methylcholanthrene is handled by the tissue in a manner analogous to that for benzpyrene. The apparent difference in local concentration of the benzpyrene derivative and the methylcholanthrene derivative is probably not real, and can be explained by the limitation of the histological technic employed by Doniach, Mottram, and Weigert. In only one, possibly minor, point does a real difference seem to exist between the two compounds. The methylcholanthrene derivative is unstable in ultraviolet light, while the benzpyrene derivative was said to be stable even upon prolonged exposure.

The question of whether the malignant transformation of cells is produced directly by the carcinogenic hydrocarbons or by some of their metabolic derivatives has been discussed at length in recent cancer literature. Berenblum and Schoental (1, 2)

in particular have held to the concept that the persistence of unchanged carcinogen is necessary to induce malignant tumors. The evidence they have adduced to support this hypothesis may be questioned, since subcutaneous tissue in contact with persistent oil suspensions of benzpyrene are continually subjected both to the parent hydrocarbon and to its slowly but continuously formed metabolic derivatives. The same authors have reported the lack of carcinogenicity of 8-hydroxy-3,4-benzpyrene and 3,4-benzpyrene-5,8-quinone, the two metabolic derivatives that they have thus far isolated and identified. Since these compounds are apparently terminal stages in the destruction of the original carcinogen, their lack of carcinogenicity does not prove that metabolic derivatives intermediate between themselves and the parent hydrocarbon are also noncarcinogenic. Two kinds of experimental evidence also tend to refute Berenblum's concept. Because both have been considered in our earlier papers we need but mention them here. (a) Methylcholanthrene applied in one dose to the skin of susceptible strains of mice produces cancer in about one-third of the animals (7, 22). The unchanged carcinogen persists in the skin for a maximum of 6 to 8 days (23), yet the ultimate malignant change may occur at least as long as 9 months thereafter. It is not probable that such a change occurs after so long an interval without the intervention of some agent other than the pure carcinogenic hydrocarbon. (b) Solutions of unaltered methylcholanthrene in anhydrous lanolin may be applied 3 times a week to mouse skin for at least 14 weeks without producing skin cancer or other apparent structural alterations (21). This cannot be reconciled with Berenblum's concept.

An alternative view has been supported at length by Weigert and Mottram (27, 28). From early studies on physically different forms of benzpyrene *in vitro* they proposed that one of the unstable forms of the carcinogen might exist for a time in association with cell lipids, and by gradual transformation into the stable form release energy for biological action. This biological action presumably was intended to include carcinogenesis. Their subsequent studies on benzpyrene in animals failed to support such a hypothesis, but did reveal that certain alkali-soluble derivatives of the carcinogen were formed in skin and other organs of the mouse. These derivatives have been further studied and related, in a working hypothesis, to benzpyrene and to the derivatives isolated by Berenblum. Because one of the primary derivatives 'BPX' accumulates chiefly at sites where benzpyrene will elicit tumors, Weigert (26) has recently made the suggestion: "Hence it is likely that its metastability and its spontaneous transformation into 'BPF' according to the laws of probability may be the reason for the stimulation of the change of a normal into a malignant cell."

The primary alkali-soluble, blue-fluorescent derivative of methylcholanthrene, which we have described, seems to share the properties of Weigert's 'BPX.' Until it is identified chemically, we shall refer to it in an analogous term, 'MCX.' It arises shortly after a single painting of the carcinogen and persists for perhaps a maximum of a month. While the contact of this substance with the tissue is several times as long as that of the unmodified methylcholanthrene, it is still but a short initial fraction of the total time that often elapses before a carcinoma arises in the skin as a result of a single painting. If the unchanged hydrocarbon is to be excluded as the direct cause of the malignant transformation, there is also good reason to rule out the blue-fluorescent, alkali-soluble derivative, for in skins that react by developing a carcinoma in response to a single application of a carcinogenic hydrocarbon a full 8 or 9 months may still intervene between the time of disappearance of the 'MCX' and the development of a clinically detectable malignant lesion.

In many cases of chemically induced cancer, and especially in that resulting from a single external application of the carcinogen (7, 22), it appears that neither the carcinogenic hydrocarbons themselves nor their recognized metabolic derivatives are present in the tissue at the time of the final stage of malignant transformation. This is cause for neither concern nor surprise. Indeed, a finding that either a particular hydrocarbon or some of its derivatives are essential at this final stage would be remarkable, when one considers the great variety of unrelated chemical compounds, biological entities such as viruses as well as physical agents like x-rays and ultraviolet light, that can induce clinically and histologically identical end results.

On the other hand, there is now considerable evidence on the role of the carcinogenic hydrocarbons, benzpyrene and methylcholanthrene, and their derivatives in the *early* stages of the carcinogenic process, especially in the skin. In this connection it is interesting to recall that photo-oxidized solutions of benzpyrene have been reported more active in producing tumors than those prepared and stored in the dark (9). Very possibly photo-oxidation may result in the formation of intermediary breakdown products similar to those that are produced in the skin of the mouse. The recent description (24) of the separation of certain of the effects of methylcholanthrene by applying it in different solvents aids in this analysis. Methylcholanthrene applied to skin in melted anhydrous lanolin produces neither a carcinogenic effect nor any

of the chemical or structural changes known to result from the use of the same compound in benzene. The lanolin solution of the carcinogen does, however, sensitize the skin so that it responds to subsequent treatments with the carcinogen in benzene by the development of cancers at a greatly accelerated rate and in a much higher percentage of animals.

Significant among the observations on lanolin-methylcholanthrene-treated skin is the finding that the carcinogen does not undergo, in the tissues, a breakdown to the blue-fluorescent, alkali-soluble compound that is seen regularly in epidermis treated with methylcholanthrene in benzene. Added to the results of the present study, these findings tend to support the general concept advanced by Weigert for benzpyrene carcinogenesis, but go beyond it in suggesting that the unaltered carcinogen may have an essential role in the earliest phase of the process, at least as the process occurs in response to methylcholanthrene.

Thus it would appear quite within the realm of possibility that the successive actions of several substances are necessary for the initiation of the carcinogenic process. Our evidence now suggests that the unchanged hydrocarbon, methylcholanthrene, prepares the epidermis for the subsequent activity of some of its derivatives. Considering the time relationships of persistence of the carcinogen and the blue-fluorescent derivative, it would seem that these substances must give way to still other agents, as yet unidentified, for the later, definitive stages of the carcinogenic process. This is, of course, merely a working hypothesis, based in part on the results of our studies and in part on purely hypothetical possibilities. Such a concept may be, but probably is not, more complex than the process that it is required to explain.

SUMMARY AND CONCLUSIONS

Fluorescence microscopic studies have been made on the skins of approximately 200 mice to which one or more applications of 20-methylcholanthrene in benzene had been given at 14 to 21 day intervals. These samples covered the full range of the carcinogenic process from beginning to end, including the final stage of many fully developed cancers. After each of the first 4 paintings, specimens were examined at intervals of a few minutes to many days; after the last painting, the longer intervals were as great as several months.

A blue-fluorescent substance appears in the epidermis within a day after a single painting with methylcholanthrene, and persists for several weeks. This fluorescence is present in fresh, unfixed, painted skin, and is not affected by formol fixation. The substance responsible for it, presumably a metabolic derivative of methylcholanthrene, is diffusely distributed in the cytoplasm of the epidermal cells, but does not appear in their nuclei. It is readily removed by extraction with weak alkali. In this and other properties it resembles the blue-fluorescent derivative of benzpyrene described by Weigert and Mottram (28) as 'BPX.' This suggests that methylcholanthrene undergoes the same sort of metabolic alterations as benzpyrene.

The absorption of methylcholanthrene by a skin that has been altered by one or more previous treatments with the same chemical is vastly different from that by the skin when it is first treated. In contrast to the selective uptake by sebaceous glands, subcutaneous fat, and the lipids in the keratin layer of the normal skin, methylcholanthrene frequently passes directly into the cytoplasm, but not into the nuclei, of the cells of the hyperplastic epidermis upon reapplication of a benzene solution. Disappearance of the carcinogen from such cells appears to be by direct metabolic transformation into a blue-fluorescent, alkali-soluble derivative, probably identical with that which appears after a single application of the carcinogen. This provides evidence of a further striking change that results from a single exposure of epithelial cells to an active carcinogenic hydrocarbon. Epithelial cell permeability is apparently significantly modified by such an exposure, so that the carcinogen in a lipid solvent can now enter freely. Further, the cells have become more resistant than normal cells to the toxic effects of the methylcholanthrene, and do not appear to be injured by the presence of the carcinogen within their cytoplasm.

Late in the carcinogenic process several structures of the skin exhibit fluorescences that do not occur in the normal skin of the mouse. The present report has been confined to the fluorescence of epithelial structures. Two general fluorescences may be distinguished, one blue and the other red. Often there appear to be varying mixtures of these two colors. The blue fluorescence observed long after the cessation of methylcholanthrene painting differs from the blue fluorescence described above chiefly in that it is not present in the fresh tissue, but appears after formol fixation and, once present, cannot be removed by alkali extraction. The red fluorescences are extremely variable, but in general appear to occur where there are also evidences of degenerative changes. Possibly they are produced in such areas by porphyrins that are liberated as a result of decomposition of hemoglobin.

The evidence on the role of the cancer-producing hydrocarbons and their metabolic derivatives in the carcinogenic process has been reviewed. How the available data may fit into a working hypothesis to account for the early phases of the reaction to carcinogens by the skin is discussed.

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Changes in Epidermal Cholesterol During Methylcholanthrene Carcinogenesis in Mice*

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The observations recorded in this paper form part of a group investigation on the changes taking place in mouse epidermis during the process of carcinogenesis induced by a standardized application of a benzene solution of methylcholanthrene. The fact that a single application of this carcinogen produces promptly a large diminution in the total lipids of the epidermis has been recorded in a previous paper (7). The present report deals with the share of total cholesterol in this diminution.

METHODS AND MATERIAL

The same strain of New Buffalo mice was used in this as in the earlier experiments. The treatment of the animals and the preparation of the samples of epidermis have been described in the previous paper. In addition to estimations of the total cholesterol content of skin treated with benzene solutions of methylcholanthrene, the skins of normal mice and of those that had received paintings with benzene alone were analyzed. The epidermis was separated from the dermis by the method described by Baumberger, Suntzeff, and Cowdry (1); the appendages, that is to say the hair follicles and the sebaceous glands, remain behind with the dermis and only the epidermis proper is removed and subjected to analysis.

As shown in the previous paper on total lipids, the amount available for analysis in one sample of skin is very small, about 5 mgm. for a normal mouse. Since the cholesterol constitutes only a small percentage of the total lipids, about 6 per cent for a normal mouse, the amount of this sterol in an individual sample of epidermis is about 0.25 mgm. For this reason each cholesterol estimation had to be carried out on a pooled sample of approximately equal areas of epidermis from several mice, as shown in the tables, so that each analysis represents the average of from 4 to 14 similarly treated animals. The tissue was minced with small scissors and extracted as previously described by repeated treatments with alcohol and ether, and then re-extracted with petroleum ether. The dry residue obtained after evaporating the solvent represents the total lipids. After being weighed it was taken up again in petroleum ether and brought to a known volume in a small volumetric flask. A measured aliquot was removed and evaporated on a hot water bath with the aid of a gentle stream of CO_2. For the cholesterol assay the residue was dissolved in chloroform and the sterol measured colorimetrically by the Burchard-Liebermann reaction. Owing to the small amount of the sterol present only the total cholesterol could be determined.

Occasionally the color obtained with the reagent was yellowish, and difficult to match with the standard. This was probably due to the methylcholanthrene itself producing a slight yellow color with the reagent. By holding down the temperature to 20 to 21° C. for color development and using a deep red filter (Wratten 29) over the ocular of the Klett-Bio colorimeter it was possible to obtain acceptable color matchings of the standard and of the sample.

RESULTS

As a suitable reference base for the amount of tissue involved the total protein nitrogen was used, for reasons given in the previous article. The ratio of total lipids in milligrams to protein nitrogen in milligrams was calculated, yielding figures of suitable size, lying roughly between 2 and 5.

TABLE I: TOTAL CHOLESTEROL IN EPIDERMIS OF NORMAL MICE

No. of animals		Cholesterol in total lipids, per cent	Mgm. cholesterol : mgm. protein N
6		3.44	0.259
8		4.89	0.247
9		5.20	0.259
14		4.12	0.210
8		4.30	0.211
10		7.74	0.362
9		8.42	0.341
Total 64	Average	5.44	0.270

Table I, which gives the results of analyses of the normal epidermis, shows that the total cholesterol

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averages 5 per cent of the total lipids. The cholesterol-protein N quotient yields, therefore, figures lying between 0.210 and 0.362, with an average of 0.270. A single application of methylcholanthrene is sufficient to lower this quotient on the fifth day after the painting. The quotient remains at this lower level for at least 10 days, and subsequent applications do not materially affect this low level except for a further slight diminution. The relevant data are recorded in Table II, which gives also the cholesterol content, ex-

TABLE II: TOTAL CHOLESTEROL IN EPIDERMIS OF MICE AFTER METHYLCHOLANTHRENE

	No. of animals	No. of applications	Killed, days after 1st treatment	Cholesterol in total lipids, per cent	Mgm. cholesterol: Mgm. protein N
Single application	5	1	2	8.04	0.282
	5	1	2	6.05	0.352
	5	1	2	7.15	0.241
	5	1	2	6.28	0.278
Total	20			Average 6.88	0.288
	9	1	3	8.27	0.232
	5	1	3	7.20	0.325
	5	1	3	7.65	0.295
	5	1	3	9.58	0.353
Total	24			Average 8.18	0.302
	7	1	5	8.37	0.236
	8	1	5	5.61	0.111
	5	1	5	6.81	0.118
	5	1	5	3.52	0.135
	4	1	5	9.08	0.206
Total	29			Average 6.68	0.161
	7	1	10	6.06	0.216
	10	1	10	7.79	0.173
	8	1	10	5.96	0.129
	8	1	10	3.28	0.107
Total	33			Average 5.77	0.171
Repeated applications	5	2	10	7.10	0.186
	5	2	10	3.72	0.095
	6	2	10	9.17	0.162
	8	2	10	5.22	0.075
Total	24			Average 6.30	0.129
	7	3	10	5.92	0.079
	5	3	10	6.53	0.204
	6	3	10	9.45	0.165
	9	3	10	6.85	0.177
	5	3	10	5.47	0.110
Total	32			Average 6.84	0.147
	5	6	14	5.37	0.145
	11	6	14	8.05	0.190
	7	6	14	10.60	0.186
	9	6	14	9.00	0.153
Total	32			Average 8.26	0.169
	8	12	30	9.70	0.166
	7	12	30	7.82	0.155
	5	12	30	5.85	0.118
Total	20			Average 7.79	0.146
	5	24	60	4.37	0.143
	8	24	60	9.65	0.134
	9	24	60	10.00	0.146
Total	22			Average 8.01	0.141

pressed as percentages of the total lipids, and the number of days that elapsed between the first application of the carcinogen and the removal of the epidermis for analysis. This removal was carried out at least 2 days after the last application, and sometimes after an even longer interval.

It is interesting to find that this change in the total cholesterol corresponds closely to the changes in the total lipids reported in our previous paper, as is shown in Fig. 1, in which the total lipid-protein N quotient and the total cholesterol-protein N quotient are plotted as ordinates against an abscissa of both weeks and the number of applications. Both the total lipids and the total cholesterol show a decided diminution on the fifth day after the first application, which persists and undergoes only a slight further decline in response to further repeated applications. While the total lipids show a diminution, the figures in Table II for the cholesterol content of the lipids indicate that their cholesterol content is slightly higher than that of the total lipids of normal skin.

Since the carcinogen is applied in a solvent, benzene, which is in itself a lipid solvent, the effect of benzene on the cholesterol content of the normal epidermis was studied in some detail. The results (Table III) reveal a striking contrast in the reaction of the epidermis to the solvent, benzene, as compared with a benzene solution of the carcinogen. There is a rapid rise in the cholesterol-protein N quotient after a single application, and this increase continues after the second and third applications. The value for the quotient diminishes after subsequent applications to figures slightly below the normal, but never reaches the low level induced by the carcinogen.

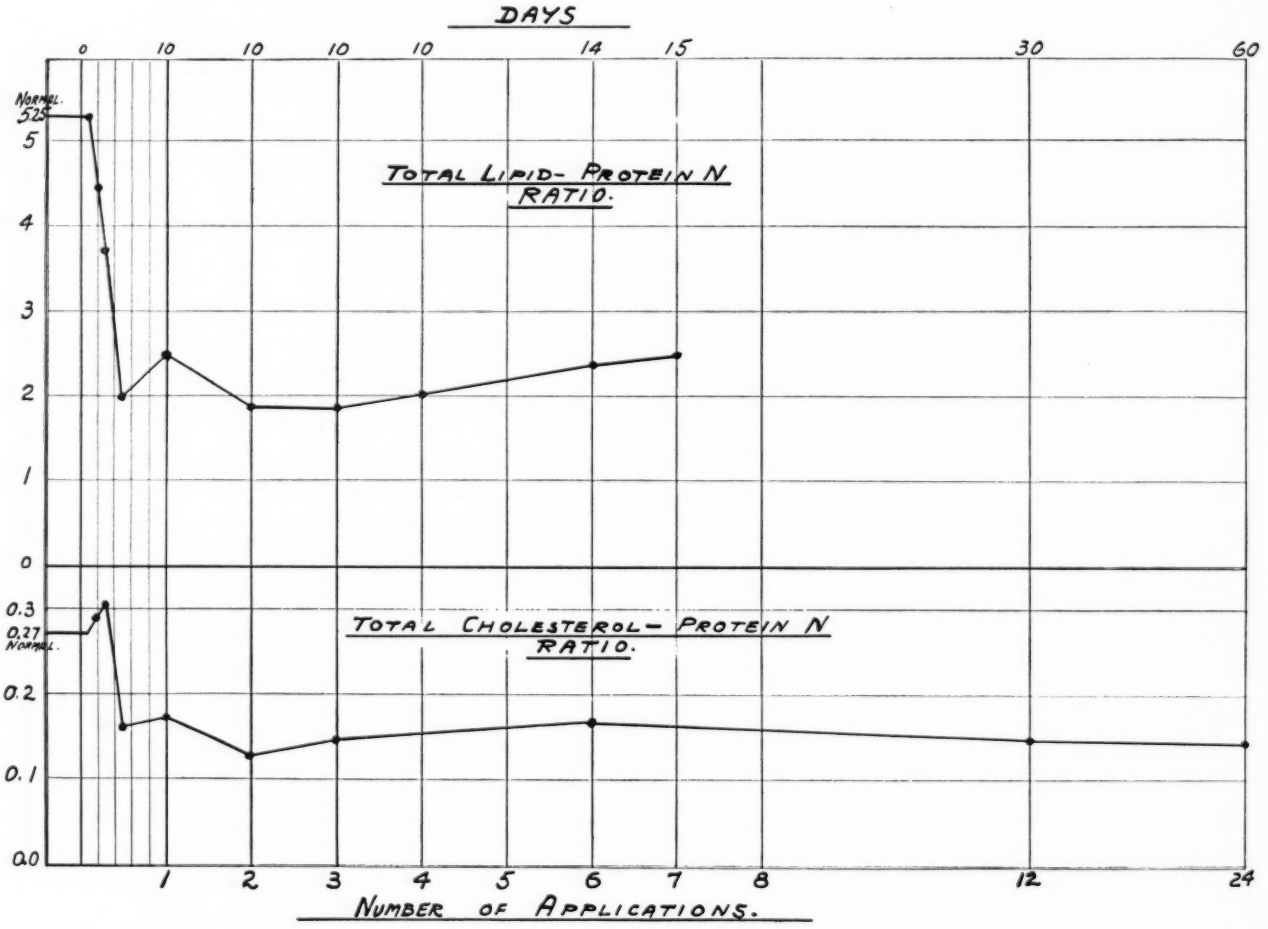

FIG. 1.—Total lipid-protein N ratio and total cholesterol-protein N ratio in epidermal methylcholanthrene carcinogenesis.

DISCUSSION

The work carried out previously by the Barnard Hospital group has established the fact that a single application of methylcholanthrene produces quickly characteristic changes in the epidermis (3). These changes are chemical and structural. There is a fall in the calcium and iron content (2); the sebaceous glands degenerate (5); and the epithelial cells, which are normally impermeable to the carcinogen, undergo a change that allows the carcinogen to go into the cytoplasm, leaving only the nucleus impermeable, as

demonstrated by fluorescence microscopy (6). To these changes can now be added a diminution in the total lipids, including the total cholesterol. There is a close time relationship between the degeneration of the sebaceous glands and the diminution in the total lipids and the total cholesterol. The glands begin to degenerate on the third day after the first application and have mostly disappeared on the fifth day, pushing out the sebum to the surface of the epidermis while undergoing disintegration. The diminution in the total lipids and in the total cholesterol sets in at just that time.

The fact that the application of benzene alone induces a striking increase can reasonably be accounted for by the functional activity of the sebaceous glands in response to removal of the sebum from the surface of the epidermis when benzene is applied to it. Eller (4) quotes Schur and Goldfarb as stating that the secretion of sebum is not continuous but stops when the skin has reached a certain degree of satura-

TABLE III: TOTAL CHOLESTEROL IN EPIDERMIS OF MICE AFTER BENZENE. CONTROLS

	No. of animals	No. of applications	Killed, days after 1st treatment	Cholesterol in total lipids, per cent	Mgm. cholesterol: Mgm. protein N
Single application	7	1	5	7.22	0.328
	9	1	5	6.46	0.558
	10	1	5	5.55	0.235
Total	26			Average 6.20	0.392
	8	1	10	7.03	0.495
	8	1	10	10.10	0.648
	9	1	10	7.25	0.344
Total	25			Average 8.11	0.495
Repeated applications	7	2	10	4.47	0.425
	8	2	10	8.45	0.520
	7	2	10	7.56	0.598
Total	22			Average 6.83	0.514
	12	3	10	6.03	0.338
	8	3	10	5.76	0.298
	5	3	10	6.40	0.381
Total	25			Average 6.06	0.339
	7	6	10	3.40	0.186
	9	6	10	3.81	0.222
Total	16			Average 3.60	0.204
	8	6	14	5.20	0.219
	9	6	14	5.44	0.308
	8	6	14	3.76	0.165
	11	6	14	3.26	0.167
Total	36			Average 4.42	0.215
	11	9	21	7.52	0.253
	10	12	30	3.58	0.173
	7	12	30	3.62	0.193
	8	12	30	3.39	0.223
Total	36			Average 3.53	0.196
	8	24	60	7.56	0.175
	10	24	60	8.74	0.275
	8	24	60	5.00	0.212
	9	24	60	4.87	0.256
Total	35			Average 6.54	0.229

tion, and that secretion sets in when the sebum is removed by washing or some other procedure. The later diminution that appears after frequently repeated application of benzene is probably due to a partial exhaustion of the glands.

It is necessary to emphasize that the results recorded in this paper and in the previous paper on lipids refer to the separated epidermis and not to the skin as a whole. In previous unpublished work analyses were carried out by one of us (L.F.W.) on the whole skin of mice treated with methylcholanthrene three times weekly for 100 days. Samples were taken at 10 day periods. No significant changes were noted in the total lipids, total cholesterol, or lipid-phosphorus of the whole skin. This point is of importance because statements exist in the literature (8) to the effect that malignant tumors of the skin have a higher cholesterol content than the normal skin; *i.e.*, dermis and epidermis. It is obvious that such a comparison between an epithelial neoplasm of the skin and the tissue from which it has arisen requires an analysis of the epithelial constituents of the normal skin. The observations recorded in this paper refer to changes in the epithelial cells preceding the development of a carcinoma. Our work, which was to have been extended to changes in the malignant cells, has been interrupted by the war.

SUMMARY

1. The total cholesterol content has been determined in the separated epidermis of normal mice and of mice that had received applications of methylcholanthrene in benzene. The values found for cholesterol are expressed as a ratio in relation to the protein N content of the epidermis. A single application was sufficient to produce a profound lowering of this ratio. Subsequent applications during the first 60 days produced only a further slight reduction.

2. A single application of benzene alone greatly increased the cholesterol-protein N ratio of the epidermis. Repeated applications of benzene diminished this ratio to values somewhat below the normal level, but higher than the values observed in skin exposed to methylcholanthrene.

3. The effect of methylcholanthrene in reducing the values for the cholesterol-protein N ratio resembled closely its effect on the total lipid-protein N ratio recorded in a previous paper.

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Further Studies on the Mode of Origin of Carcinomas of the Skin*

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In a previous paper (4) I described a series of early solitary squamous cell carcinomas of the skin, which showed (a) the frequent presence of a marginal zone of hyperplastic epidermis around the growths; (b) the absence of demarcation between this hyperplastic and the neoplastic epithelium, the one passing gently into the other; (c) the frequent retention of epidermal stratification and regular papillation in the growths; and (d) the occasional presence of demonstrably separate multiple foci of active carcinoma springing from a considerable field of hyperplastic or neoplastic epidermis. These structural features were incompatible with the prevalent view that these tumors had arisen each from a single minute focus at a single point in time and had enlarged solely by proliferation; they could be explained only as the result of progressive neoplastic change spreading from one or more focal points over a particular field of epidermis, which in the localized growths studied often had a diameter of at least 1.5 or 2 cm.

The present paper describes a similar study of some other types of epidermal and allied carcinoma, examined for evidence of their mode of origin. These comprised (a) early localized nonulcerated squamous cell carcinomas of the lip or tongue; (b) extensive, sometimes multiple, superficial squamous cell carcinomas of the skin; (c) basal cell carcinomas of the skin. Only selected examples of each of these are described, but the features illustrated were observed in many other tumors also. The methods used were as stated in my earlier paper (4). The lip tumors were all sectioned in a sagittal plane.

A. EARLY LOCALIZED SQUAMOUS CELL CARCINOMAS OF THE LIP OR TONGUE

Case I. (Section No. 4468)

The growth presented as a slightly raised rough area about 5 mm. in diameter on the lip of a man of 45 (Fig. 1).

The epithelium.—The lesion is an early nonulcerated highly differentiated epidermoid carcinoma, penetrating the subjacent tissues to a depth of less than 2 mm. The downgrowths show all the characters of epidermis except that no definite stratum granulosum is present; each shows an abundant basal cell layer, passing into a well differentiated layer of spinous cells, then a keratinized core demonstrably continuous with the heavily keratinized stratum corneum on the surface. The basal cell margins of some of the downgrowths are a little frayed out and mingled with the abundant leukocytic infiltration in the surrounding stroma. Toward the outer (cutaneous) margin of the tumor the epidermis undergoes steady thickening, and becomes imperceptibly continuous with the epithelium of the tumor (near X). Toward the inner margin, however, the mucosal epithelium shows little increase in thickness and its junction with the neoplastic epithelium (at Y) is abrupt.

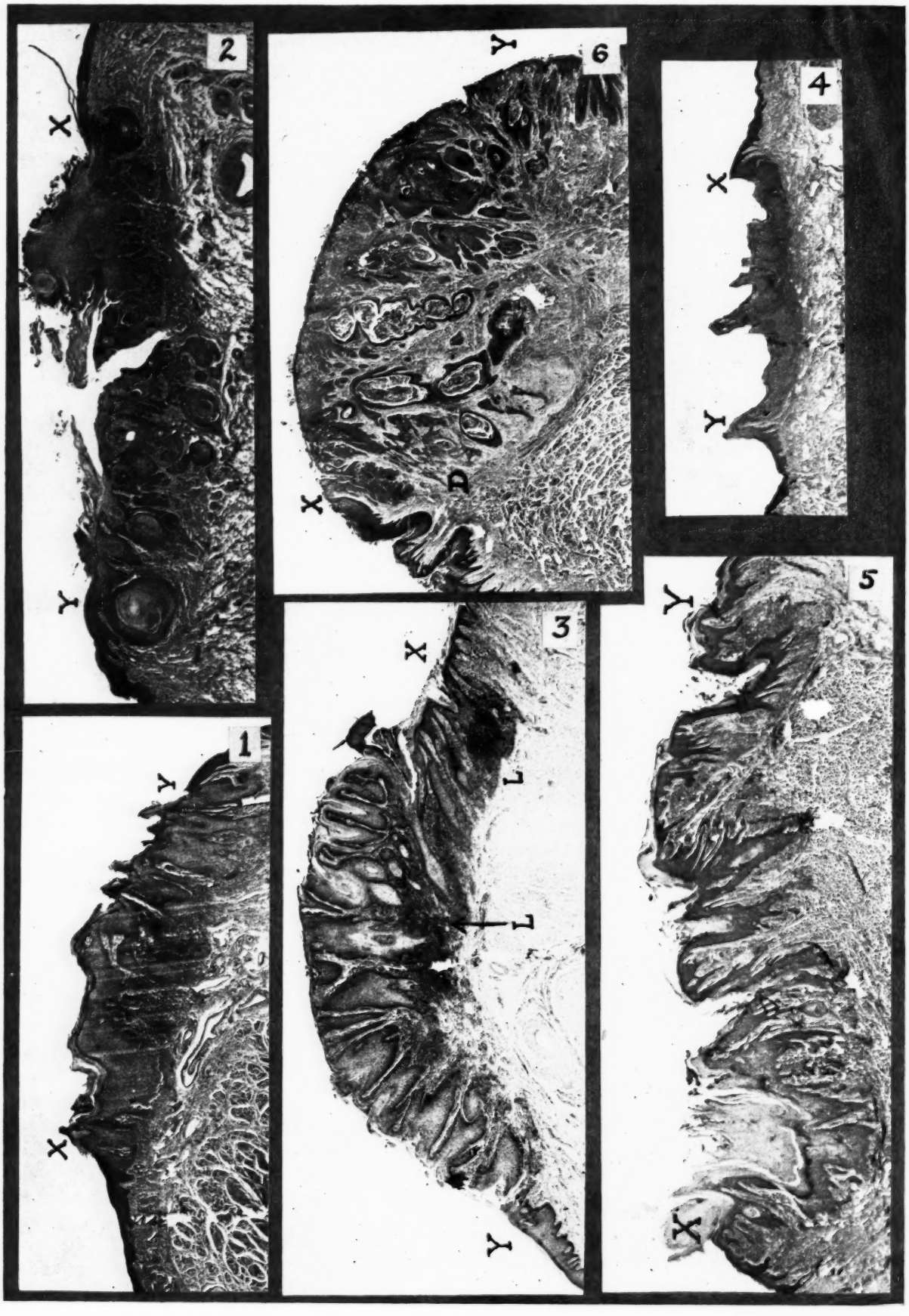
Stromal changes.—Leukocytic infiltration, mainly by lymphocytes, is abundant in the tissues immediately beneath the cancerous epithelium and the marginal zone of hyperplastic epidermis, but is absent from the tissues beneath the unaltered mucosal epithelium. The dermis extending from the outer margin of the growth shows notable increase of elastic fibers, which are enlarged, and in places show homogeneous fusion, forming in the hematoxylin-stained tissue a prominent bluish layer measuring about 0.5 mm. in thickness. Residual patches of this altered elastic tissue are present beneath parts of the carcinomatous downgrowths. The subepithelial mucosal tissue shows no sign of increased elastic tissue.

Case II. (Section No. 9025)

The lesion was a slightly thickened incrusted area about 8 mm. in diameter on the lower lip of an elderly man (Fig. 2).

The epithelium.—The growth is a well differentiated epidermoid carcinoma with irregular or rounded downgrowths with central concentrically laminated cornification. The downgrowths have penetrated to a nearly uniform depth of about 2 mm., the depth of penetration being slightly greater at the central than at the peripheral parts of the growth. Mitotic figures are few. Although the limits of the neoplasm are

* Because of the difficulties of international communication the author has not read proof of this article.



FIGS. 1-6

fairly well defined, there is no sharp demarcation between neoplastic and nonneoplastic epithelium. The surrounding epidermis, which is atrophic and devoid of papillae, undergoes slight uniform increase in thickness as it nears the tumor; and the thickened epidermis passes into cancerous epidermis by a fairly rapid, but not abrupt, transition (at X). In the transition from epidermis to tumor the stratum granulosum is lost, and the stratum corneum becomes thicker and paler staining. On the mucosal side of the tumor the epithelium shows only slight hyperplasia, and the transition from it to tumor (at Y) is also rapid, but not abrupt.

Stromal changes.—There is moderate lymphocytic infiltration in the tissues around the tumor downgrowths and deep to the immediately adjacent mucosal epithelium and epidermis. The elastic tissue in both the dermis and the subepithelial tissue of the mucosa shows moderate increase, and appears in the hematoxylin-stained section as an incomplete bluish zone of swollen fibers, more prominent on the cutaneous than on the mucosal side of the tumor.

Case III. (Section No. 2168)

A nonulcerated hemispherical growth about 1 cm. in diameter was excised from the lip of a man of 41 (Fig. 3).

The epithelium.—The growth is an active but fairly well differentiated epidermoid carcinoma, with distinct differences of structure, however, in its cutaneous and mucosal halves. As Fig. 3 shows, its cutaneous half has a highly keratinized surface, the downgrowths from which also contain well developed keratin cores; and in many parts it shows a well marked stratum granulosum with coarse keratohyalin granules. The mucosal half of the tumor, on the contrary, shows only slight surface keratinization, only poorly keratinized centers in its downgrowths, and no sign of a stratum granulosum. In general, also, the downgrowths of the cutaneous half are more slender and pointed, and have penetrated deeper than those of the mucosal half, the downgrowths of which are broader, more obtuse, and more regularly spaced. Mitotic figures are plentiful in the cancerous epithelium; they appear to be about equal in number in the two halves of the growth and an unusually high proportion of them are in the diaster phase. The epithelium marginal to the tumor on both its mucosal and cutaneous sides shows a zone of hyperplastic thickening, which passes quite gradually into the neoplastic tissue. The width of the zone of epidermal hyperplasia (about X) is greater than that of the mucosal hyperplasia (about Y), and the transition from hyperplasia to neoplasia on the epidermal side is more gradual than on the mucosal side.

Stromal changes.—As can be seen at LL, there is a heavy infiltration of leukocytes in the connective tissues deep to the growth; this consists of mingled lymphocytes and plasma cells. It is more abundant under the cutaneous half of the tumor than under the mucosal half, and while many leukocytes are also collected deep to the hyperplastic epidermal zone there are very few beneath the zone of mucosal hyperplasia. The dermal elastica peripheral to the tumor shows a definite increase, and in hematoxylin-stained sections forms a prominent bluish layer; but there is no evidence of increased elastic tissue on the mucosal side of the growth.

Case IV. (Section No. 1995)

An incrusted projecting growth of 12 months' duration and 8 mm. in diameter was excised from the lower lip of a man aged 76.

The epithelium.—The hemispherical tumor shows both bulky and slender anastomosing downgrowths with a high degree of epidermoid differentiation, showing well formed spinous cells, stratum granulosum, and abundant keratinization with many concentrically laminated epithelial pearls. Mitotic figures are few in the cells of the more bulky heavily keratinized masses, but more numerous in the slender less keratinized epithelial strands. The tumor penetrates almost to the muscle of the lip and has a maximum vertical depth of 4 mm. As the epidermis approaches the tumor it shows some irregular thickening, and it is impossible to decide whether the marginal downgrowths belong to the tumor or are merely hyperplastic. The epithelium of the mucosa shows little or no thickening as it approaches the tumor, and there is an abrupt transition from mucosal epithelium to tumor.

Stromal changes.—A dense collection of lymphocytes with some plasma cells underlies the tumor and the immediately adjoining epithelium. The dermal elastica shows great increase and degeneration, appearing in the hematoxylin-stained section as a prominent, pale blue, almost homogeneous layer measuring up to nearly 2 mm. thick and extending right to the edge of the excised tissue. There is no increase of the elastic tissue in the mucosa of the lip.

DESCRIPTION OF FIGURES 1 TO 6

Figs. 1 to 5.—Sagittal sections of lip tumors; the epidermal and mucosal sides marked X and Y respectively. Mag., Figs. 1 and 2, × 14; Fig. 3, × 10; Fig. 4, × 6; and Fig. 5, × 12.

Fig. 6.—Carcinoma of tongue, margins of which are near X and Y. Mag. × 6.

Case V. (Section No. 4788)

A heavily cornified conical growth 1 cm. in diameter was excised from the lower lip of an elderly man. Fig. 4 depicts its vertical section after detachment of the mass of horny material (Fig. 4).

The epithelium in the base of the crater left by detachment of the keratin is highly differentiated and epidermis-like, except that there is no distinct stratum granulosum. It consists of broad, blunt downgrowths, which have penetrated to a uniform depth of about 2 mm. The epithelium marginal to the tumor on both its cutaneous and mucosal sides shows a zone of hyperplastic thickening about 3 mm. wide, and this hyperplastic epithelium is gently continuous with the cancerous epithelium at the crater lips XY.

Stromal changes.—Immediately beneath the tumor there is an abundant collection of lymphocytes, but very few beneath the zones of epithelial hyperplasia. Both the dermis and mucosal connective tissue marginal to the tumor show extensive and prominent increase and degeneration of elastic tissue. The tumor downgrowths partly interrupt the layer of altered elastica.

Case VI. (Section No. 8891)

A man aged 70 had a growth excised from his lip 2½ years previously. This had recurred 6 months later, and had been treated by irradiation. It had again recurred recently and been excised. The excised tumor consisted of a flat thickening with a slightly raised rough surface, measuring 12 mm. in diameter (Fig. 5).

The epithelium.—The growth consists of a thickened, irregularly folded, and papillated layer of perfectly differentiated epidermis-like tissue, with well formed spinous cells, prominent, almost uninterrupted stratum granulosum, and sharply demarcated stratum corneum. But for its invasive power, very apparent from its having penetrated into the muscle, this epithelium shows no histological evidence of malignancy. Mitotic figures are very few. The epidermis marginal to the growth shows gradual hyperplastic thickening and exaggerated papillation as it approaches the tumor, and it is impossible to determine where, in the neighborhood X, nonneoplastic epidermis ends and neoplastic epidermis begins. Although the epithelium of the mucosa near the tumor shows little hyperplastic thickening, here also it is impossible to say where, in the neighborhood Y, normal epithelium ends and tumor begins. The mucosal epithelium has a very prominent stratum granulosum, which passes uninterruptedly into that of the cancerous epithelium.

Stromal changes.—Dense collections of lymphocytes lie beneath the epithelium of the growth and of the immediately adjacent skin and mucosa. The dermal elastic tissue is greatly increased, forming a prominent stratum up to 2 mm. thick and showing swelling and hyaline fusion of its fibers. Elastic tissue in the mucosa is not increased.

Case VII. (Section No. 4430)

For 12 months a man aged 69 had noticed a thickened white patch on the side of his tongue. This was excised, and showed on section a mass of white infiltration 1.5 cm. in diameter and 8 mm. in depth (Fig. 6).

The epithelium.—The growth is a well differentiated, cornifying epidermoid carcinoma, parts of which, however, as at D, show fine anastomosing downgrowths with many mitotic figures. The neighboring epithelium on the side of the growth, X, shows gradual thickening and increased papillation as it approaches the tumor; at X it is continuous, without clear demarcation, with the cancerous epithelium. On the opposite side of the tumor, Y, it is impossible to decide even approximately where noncancerous epithelium ends and cancerous begins. The tumor between X and Y has a complete epithelial surface, from which the rest of the tumor appears as downgrowths.

Stromal changes.—The downgrowths have penetrated into the muscle, causing atrophy of its fibers and considerable fibrosis, accompanied by large collections of leukocytes including lymphocytes, plasma cells, and polymorphonuclear cells. There is no great change in the elastic tissue.

Comment on Group A

The structure of these 7 specimens, like that of the skin tumors previously described, does not accord with a simple unicentric mode of origin, but shows a process of spreading cancerization of a considerable, though restricted, epithelial field. As in the skin tumors, so here, there is the same marginal hyperplasia of the epithelium as it approaches the growth, the same gentle transition of hyperplastic to neoplastic epithelium without any demarcation, the same general preservation in the growths of the normal architectural pattern of the epithelium as regards stratification and papillation, and in some specimens (*e.g.*, V) the same crater-like invagination of the neoplastic epithelium into the underlying tissues. In some of the lip tumors (*e.g.*, I and IV) there is structural evidence that cancerous change was still progressing in the adjacent epidermis but not in the mucosal epithelium. The greater prominence of stromal changes in the dermis than in the mucosa near the growths (I, II, III, IV, and VI) also suggests that in many cases more is contributed to the tumors by the epidermis than by the mucosal epithelium.

That both epidermis and mucosal epithelium do contribute, however, is shown clearly by tumor III, in which the outer and inner halves show distinctly different epithelial characters, resembling those of epidermis and mucosa respectively. It is noteworthy, also, that this specimen shows changes in the neighboring mucosal elastic tissue as prominent as in the neighboring dermal elastic tissue. This specimen shows unmistakably that the growth has arisen, and is still arising, by progressive cancerous change *in situ*, in an epithelial field at least 1 cm. in diameter, which includes both the epidermis and the mucous membrane of the lip.

Tumor VI also deserves special comment. This was the third recurrence of a growth treated first by excision and later by radium. Yet it consists of an entirely superficial layer of perfectly differentiated epidermis-like epithelium, which shows gentle transition to normal epithelium on both the epidermal and mucosal sides. The structure cannot be referred to proliferation and extension of growth from a small postoperative residuum of the original tumor, but has all the appearances of origin *in situ* from a considerable field of epithelium. The recurrence was a result of incomplete removal, not of the original neoplastic mass, but of the original potentially cancerous field; a residual part of this field has become cancerous in its turn.

I must add here that only after I had completed my own study of the genesis of carcinomas of the lip did I read Lohmer's excellent account (2) of the same subject written in 1900. Lohmer's findings are similar to my own; he found the tumors to consist of downgrowths of the intact surface epithelium; and to show "a gradual transition of the surrounding normal epidermis into the tumor."

B. EXTENSIVE SUPERFICIAL SQUAMOUS CELL CARCINOMAS OF THE SKIN

Case VIII. (Section Nos. 3468 and 3502)

A woman aged 64 had had varicose veins and chronic ulcers of both legs for 20 years. For 6 months lumpy growths had been developing on the right crural ulcer. That these were cancerous was shown by biopsy, and the leg was amputated.

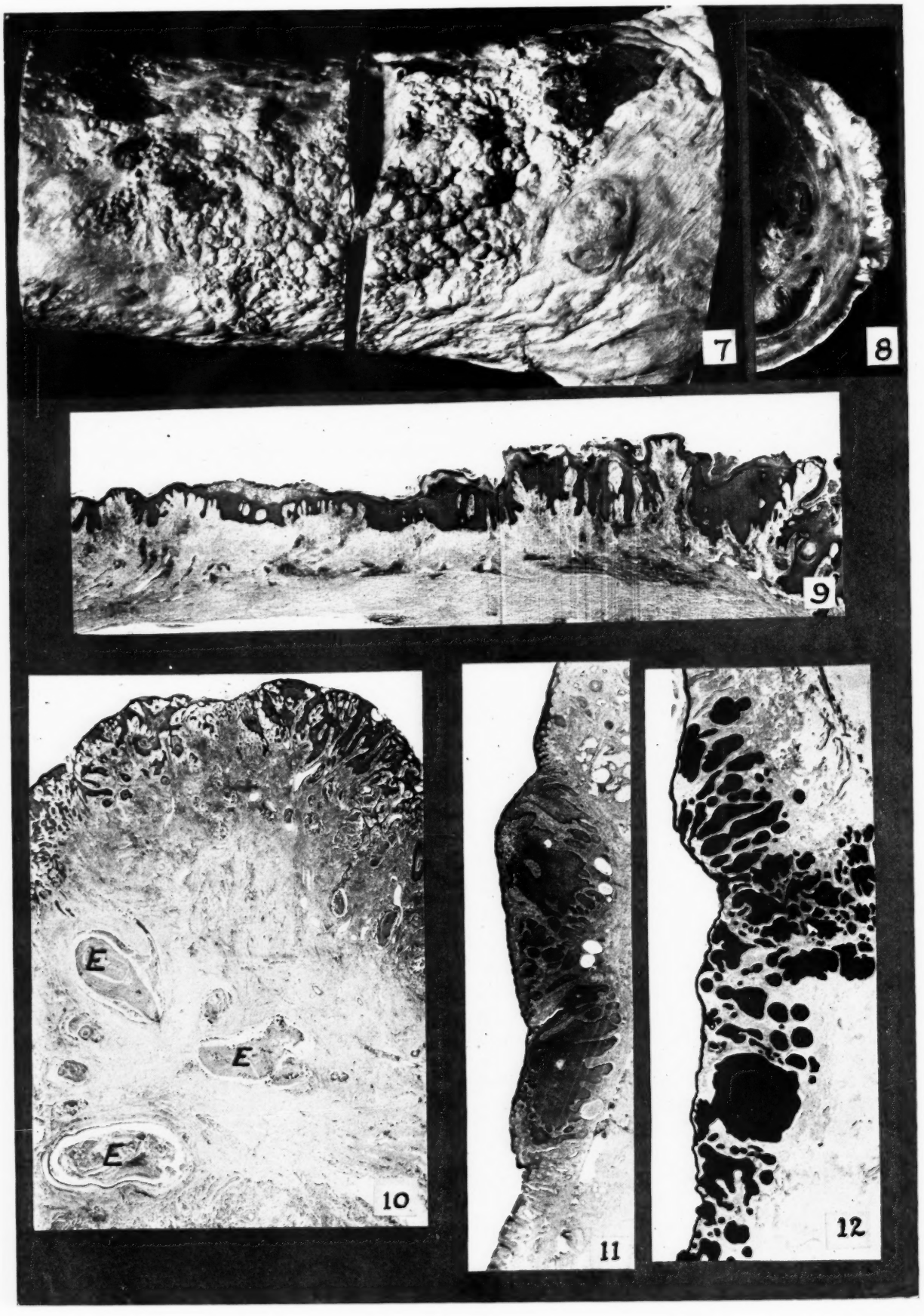
Naked-eye description.—(Fig. 7). The skin of the anterior and medial aspects of the leg was largely replaced by an irregularly nodular, slightly raised layer of growth. The area covered by obvious growth measured 15 cm. long and 8 cm. in main width; but the margins of this area were ill defined, the nodular tumor becoming gently continuous with the surrounding noncancerous skin over a zone 1 to 2 cm. wide. Several small areas of superficial ulceration of the nodular growth were present, and near its center there was a deeper area of ulceration with undermined edges and an irregular sinus about 2 cm. deep. Dissection of the soft tissues off the bones showed these to be uninvolved. Serial horizontal sections of the soft tissues at planes 1 cm. apart were made (Fig. 8), and these clearly displayed the extensive superficial layer of white growth and its gentle continuity with the unaffected epidermis. Microscopic sections were prepared from all the horizontal slabs.

Microscopic description. (a) *The epithelium.*—(Fig. 9). The tumor consists of a thick, irregular layer of perfectly differentiated epidermis-like epithelium clothing the thickened, scarred dermis. In all situations around the margins the limits of the cancerous epithelium cannot be decided even approximately; the surrounding unaffected epidermis becomes gradually thicker as it approaches the growth, and this thickened epidermis becomes gently continuous with that of the growth. Epithelialization of the cancerous area is nearly complete, only small areas of naked granulation tissue being present here and there. Penetration of the thickened dermis by growth has occurred only in the region of the deep sinus referred to, the irregular granulating walls of which are partly lined by a thick layer of cancerous epithelium, nearly 1 cm. thick in places and situated at a maximum depth of 2 cm. below the surface. (b) *The stroma.*—The dermis and subcutaneous tissue have been converted into a thick layer of dense fibrous tissue surmounted in most places by young vascular granulation tissue, on which the epithelium lies. Elastic tissue stains show only scattered, coarse fibers in the thickened and scarred dermis. Collections of leukocytes of all kinds are plentiful in the connective tissues in most places.

Case IX. (Necropsy No. 6721)

History.—A man 55 years old gave the following history: When aged 20 he had had a severe burn of the left upper limb and the side of the chest. The resulting scars had remained sound until about 2 years ago, when the scar on the forearm developed spreading ulceration. When admitted to hospital, he presented a large superficial ulcer 15 cm. in main diameter, on the outer aspect of the elbow and upper forearm, with irregular, raised edges and a dirty, granular base. A diagnosis of Marjolin's ulcer was made, and excision down to the deep fascia with a small margin of healthy skin was performed in September, 1939.

Microscopic examination.—Sections from the margins of the ulcer show greatly thickened, chronically inflamed dermis, surmounted and superficially invaded by irregularly nodular carcinomatous tissue of well differentiated, cornifying epidermoid type, but



FIGS 7-12

with plentiful mitotic figures in the cellular outer parts of the epithelial clumps. At the ulcer margin the carcinomatous tissue is heaped up to form an irregular rampart nearly 1 cm. deep in places. The junction of this with the surrounding epidermis is fairly well defined, with a rapid, but not abrupt, transition from noncancerous to cancerous epithelium. The excision line appears to be well clear of the growth, the marginal zone of noncancerous epidermis measuring about 1 cm. wide. Sections from the floor of the ulcer show mingled cancerous tissue and poorly organized, inflamed granulation tissue, with an underlying layer of thickened dermis which is nowhere found to be penetrated to its deepest part by carcinomatous growth. Thus complete removal of the growth appeared to have been achieved.

Subsequent history.—Skin grafting of the denuded area was attempted on several occasions during the next 3 months, but without success. The area of ulceration extended, and in December, 1939, several smaller separate areas of ulceration developed in parts of the scars above the elbow. These steadily enlarged, deep x-ray therapy failed to improve the condition, and the patient died of axillary cellulitis and bronchopneumonia in March, 1941.

Necropsy.—The large ulcer on the forearm extended from the elbow to the wrist and measured 20 cm. long and 6 cm. wide. Its base was smooth and formed by the muscles and fasciae, its margins irregularly nodular and evidently cancerous in many parts. In the cubital fossa there was a superficial circular area of ulceration 4 cm. in diameter, with nodular base and margins, separated from the large ulcer by 2 cm. of intact, scarred skin. A third area of ulceration, also nearly circular, and about 4 cm. in diameter, was present on the anterior aspect of the middle of the arm, and several smaller separate patches of ulceration were present in the scarred skin in the same region. No axillary or visceral metastases were found.

Microscopic examination of necropsy tissues.—(a) Sections from both the upper and lower ends of the large ulcer of the forearm show cancerous tissue with raised margins similar to that described above, and the base of the ulcer in its peripheral part also shows patchy cancerous infiltration. The marginal epidermis shows hyperplastic thickening as it approaches the growth, and becomes continuous with the cancerous epithelium. (b) Sections from the smaller ulcers on the arm show similar characters. (c) Sections of intervening skin prove that the several cancerous areas are separate and multiple.

CASE X. (SECTIONS NOS. 8139, 8216, 8235, ETC.)

History.—On admission to hospital in June, 1936, an otherwise healthy young man of 28 gave the following story: At the age of 4 he had had "abscesses" of the right calf, right arm, and left knee. These were called "actinomycosis" by his doctor, who incised and packed them. The calf and arm healed in about a year, but the knee failed to heal. An unsuccessful attempt at skin grafting was therefore made at the age of 5, after which there was always an ulcer except for brief periods of a few weeks, when temporary healing took place on two or three occasions. The ulcer was never painful. It had been treated by many doctors without benefit. During the first 6 months of 1936 it had doubled in size.

Examination showed thin scarred areas, each about 5×2.5 cm., on the back of the right arm and the right calf. Below the front of the left knee there was a large, irregular ulcer about 7 cm. in main diameter, with undermined edges, and everted hypertrophic lower edge, and red, cauliflower-like granulations in the base, which was separated from the surface of the tibia by a centimeter or more of firm soft tissue. The Wassermann test gave a negative result. Two fragments of tissue, one from the lower edge of the ulcer, and one from its center, both showed squamous cell carcinoma. The ulcer was excised, along with a margin of one-quarter of an inch of tissue from its edge; when it was dissected off the bone it was found to be adherent to this only at one small area. Wolff and Thiersch grafts were applied.

Microscopic examination.—(a) A vertical section from the center of the ulcer is shown in Fig. 10, from which it will be seen that the cauliflower-like granulations observed in this situation are clothed by an irregular layer of carcinomatous epidermis, which penetrates into the tissues to an average depth of about 5 mm. These tissues do indeed show the characters of organizing granulation tissue, which, however, is epithelialized and invaded by cancerous epithelium. Deeper in the tissues, and apparently unrelated to the surface epithelium, lie large epithelial formations, EEE, the deepest of which lies nearly

DESCRIPTION OF FIGURES 7 TO 12

FIGS. 7 to 9.—Cancerous varicose ulcer. Fig. 7 is a general view after amputation; the transverse cleft is the site of removal of tissue for preliminary sections. Fig. 8 is a sectional view of one of the horizontal slices. Fig. 9 is a micro-section of a marginal part of the growth. Mag. $\times 7$.

FIG. 10.—Cancerous granulations in chronic ulcer. Mag. $\times 6$.

FIG. 11.—Basal cell growth of eyelid. Mag. $\times 12$.

FIG. 12.—Basal cell growth of chin. About three-quarters of the growth shown in silhouette. Mag. $\times 9$.

2 cm. below the surface and about 5 mm. from the deep surface of the tissues where these were stripped off the tibia. These formations consist of masses of keratin partly surrounded by a thin inactive-looking epithelial investment and partly by abundant collections of foreign body giant cells. The keratin masses themselves show signs of early calcification. (b) A piece of thickened periosteum, removed from the place where the ulcer adhered to the tibia, shows no evidence of growth. (c) A vertical section through the upper edge of the ulcer and the accompanying margin of tissue shows in a centripetal direction: (i) A zone 5 mm. wide of noncancerous, slightly thickened epidermis, continuous with (ii) a zone 2.5 mm. wide of cancerous epithelium clothing inflamed granulation tissue as in Fig. 10, and penetrating to a depth of 2 mm. (iii) a central zone of inflamed granulation tissue devoid of any epithelium. (d) A vertical section at the lower part of the lesion shows a zone of gentle transition from probably hyperplastic to probably neoplastic epithelium, in which it is impossible to specify the limits of either.

Subsequent progress.—The grafts applied after excision of the ulcer failed to take. Thiersch grafting was again attempted in August, when microscopic examination of biopsy fragments from the edge and center of the area showed carcinomatous tissue in the former but only granulation tissue in the latter. Further biopsy fragments were examined in September; 2 pieces from the edge showed granulation tissue only, 1 from the center showed carcinoma. Repeated skin grafts were tried in the ensuing few months, mainly without success. Biopsy fragments in January and in February, 1937, both showed carcinoma. In March diathermy excision of the unhealed area, measuring 5×6.5 cm., was performed. In May the wound was clean and nearly healed, and the patient went home. He was readmitted in June with a fungating mass 2×1 cm. in the center of the otherwise healed scarred area. This was excised down to the periosteum, and found microscopically to be carcinomatous. The wound healed well, and the patient was discharged in July, 1937. A letter in April, 1943, stated, that "After leaving hospital in 1937 my leg healed in about four months, and remained so until about 12 months ago when I knocked it and since then I have not been able to get it to heal," but that "up to the present there has been no sign of that trouble recurring."

Comment on Group B

Each of these 3 cases shows extensive superficial carcinoma supervening on long standing, previous inflammatory disease. In each the sequence of events and the microscopic findings are alike incompatible with a simple unifocal origin, but point clearly to widespread cancerous change in the epidermis.

In Case VIII the whole area of previous varicose ulceration became rapidly clothed by a layer of cancerous epidermis in gentle continuity with the surrounding normal epidermis. This rapid cancerous epithelialization can be plausibly interpreted in only two ways: (a) the formerly ulcerated area may have recently become epithelialized and the epithelium may then have become cancerous *in situ* over the whole area; or (b) epithelialization may have been effected by already cancerous epidermis that grew from, and is still growing from, the epidermis around the entire periphery of the former ulcer. Of these alternatives, either of which involves the assumption of widespread cancerous conversion of epithelium, the second appears much the more likely. This view, then, points to the interesting conclusion that cancerous epithelium, doubtless by virtue of its more vigorous powers of growth, is able to epithelialize rapidly the surface of a large chronic ulcer that the normal epithelium has failed to clothe.

Case IX also affords proof of the widespread origin of the cancerous change in the epidermis. Excision of the large cancerous ulcer was found microscopically to be apparently adequate and complete. Yet within a few months many parts of the margins around the huge excisional ulcer showed microscopically proved recurrent growth, and multiple separate areas of cancerous change had also developed. Clearly, recurrence here was due, not to inadequate removal of the initial area of growth, but to continued cancerization in an extensive field of predisposed epidermis. The appropriate surgery in such a case was amputation well above the scarred area.

Case X also exhibited repeated recurrences following apparently complete surgical removals of the growth. Here, however, the predisposing lesion was much less extensive than in Case IX, and the recurrent growths were much more localized. There is therefore reason to hope that the last excision may now have eradicated all the predisposed tissue, though this cannot yet be assumed certain.

It remains to add here that there are special instances of widespread superficial squamous cell carcinoma of the epidermis other than those supervening on scars and ulcers. Bowen's disease, formerly called a "precancerous dermatosis," is now regarded as genuine carcinoma *in situ*. I have several specimens of this disease, study of which shows clearly that the cancerous change is taking place within the epidermis over extensive, often multiple, areas. Similar widespread changes are to be seen in vulval leukoplakia, which also often gives origin to extensive or multiple invading carcinoma. X-ray and radium dermatitis carries similar risks. In all these

lesions the entire areas affected must be regarded as fields of potential (or actual) cancerous change.

In this connection attention must be drawn to Hauser's excellent paper (1), published as long ago as 1897. In this he strongly combated Ribbert's ill-founded views on the histogenesis of carcinoma, and described a case of recurrent carcinoma of the vulva that presented clear evidence of progressive cancerous change in the epidermis and in which, therefore, recurrence of the growth had arisen, not from residues of the original tumor, but from fresh areas of the unstable epidermis, as taught earlier by Thiersch.

C. BASAL CELL CARCINOMAS OF THE SKIN

Basal cell, or rodent, carcinoma is a very common disease in Australia; and, in spite of the increasing use of radiation in preference to excision, I have had the opportunity of studying nearly 200 excised growths of this kind. Many of these afforded evidence of multicentric and progressive origin; I select only 4 examples for brief description.

Case XI. (Section No. D20)

A flat plaque-like thickening of the skin was excised from the lower eyelid of a middle-aged man (Fig. 11).

Histology.—The growth is a typical basal cell carcinoma, 7 mm. in diameter, consisting of multiple irregular downgrowths from the epidermis to a fairly uniform depth of about 1.5 mm. The growth has a complete epithelial surface, and it is impossible to say whether this is neoplastic or consists of the residual superficial layers of the epidermis. At the margins the junction of cancerous epithelium with surrounding noncancerous epidermis is abrupt, but the epidermis shows some thickening and increased papillation as it approaches the tumor. The deepest parts of the downgrowths contain some small cysts, clearly derived from obstructed sweat glands and partly lined by flattened sweat duct epithelium. The dermis deep to the downgrowths shows collections of lymphocytes, plasma cells, and polynuclear leukocytes, and the dermal elastic tissue near the growth shows moderate increase and swelling of its fibers.

Case XII. (Section No. 4960)

A flat plaque-like thickening of the skin was excised from the chin of a woman aged 58 years (Fig. 12).

Histology.—The growth is a typical basal cell carcinoma, 1.5 cm. in diameter, consisting of epithelial downgrowths that penetrate the subjacent tissue to a maximum depth of 3.5 mm. The surface of the tumor is clothed by a layer of unaltered epidermis, with which some of the tumor downgrowths are connected by broad or narrow bases. Other downgrowths are unattached to the surface epithelium but are connected with, or envelop, hair sheaths and follicles. In some of the hair follicles of the skin marginal to the tumor there are small circumscribed groups of altered cells identical in appearance with those of the main growth. The dermis shows collections of lymphocytes and plasma cells around the tumor clumps, and there are prominent thickening and degeneration of the dermal elastic tissue around the tumor, extending without diminution to the edges of the excised tissue.

Case XIII. (Section No. 5308)

During a period of 12 years an elderly man had developed 11 separate small, flat, nonulcerated skin tumors, 5 on the back, 2 on the chest, 2 on the neck, 1 on the shoulder, and 1 on the lip. One of these was excised from the back.

Histology.—The tumor measures 6 mm. in diameter and about 1 mm. in depth. It consists of some small, solid, epithelial clumps with the typical structure of basal cell carcinoma, and some larger clumps with central cysts. Most of the clumps are connected with the intact surface epidermis or with the necks of sebaceous glands or hair sheaths close to the surface. The surrounding epidermis shows some hyperplastic thickening as it approaches the growth; and at S, at a distance of 2.5 mm. from the edge of the main tumor, the basal layer is replaced by a separate small focus of definite basal cell carcinoma with commencing downgrowths. The dermis deep to and for some distance around the main growth contains collections of lymphocytes and many young fibroblasts, which are also plentiful immediately beneath the satellite focus S. The dermal elastic tissue is swollen to a thick irregular bluish zone in the hematoxylin-stained section; this is most conspicuous beneath and close to the main growth and also beneath S, and it is less pronounced towards the edges of the excised tissue (Fig. 13).

Case XIV. (Section No. D3205)

(This specimen was given to me by Colonel W. B. Wartman, of the U. S. Fourth General Hospital, when stationed in Melbourne. To him my grateful acknowledgment is made.)

For many years a man about 55 years old had had many small, indolent skin lesions, which had grown slowly and become more numerous. Examination showed many fat, slightly irregular thickenings of the skin ranging from sizes barely visible up to 1 cm. in diameter, scattered over the back, chest, elbows, and face, some of the largest with early ulceration. A patch of small lesions was excised for examination.

Histology.—The excised strip of skin, about 1.5 cm. long, contains multiple separate tiny basal cell carcinomas springing directly from the basal layer of the epidermis, to which they are broadly attached or which they partly replace. Within a distance of 1 cm. the section shows at least 8 of these minute separate growths. The largest has penetrated the dermis to a depth of 1 mm.; the others, such as those shown in Fig. 14, have penetrated less than 0.5 mm. All have the typical structure of solid basal cell carcinoma. The dermis contains collections of lymphocytes and young fibroblasts, but there is little visible change in the elastic tissue.

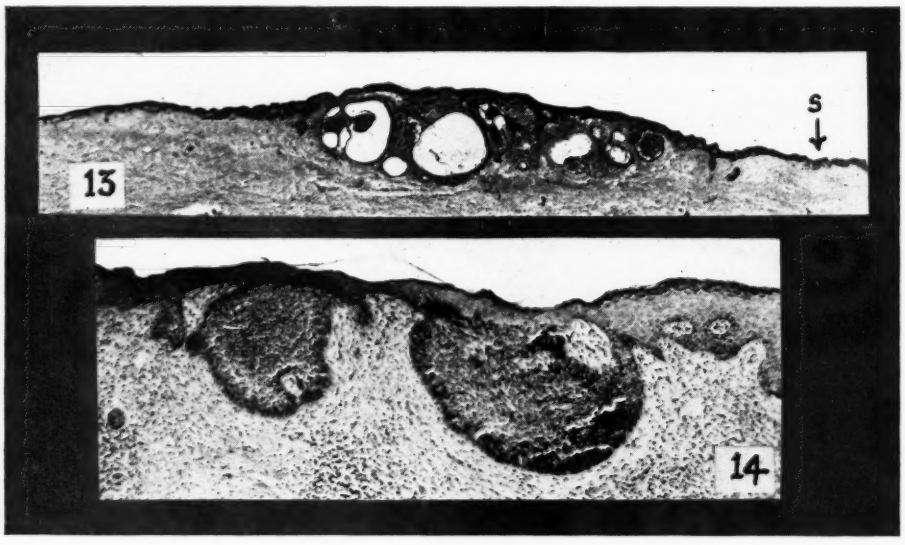


FIG. 13.—Basal cell growth of back. S denotes small separate focus of growth. Mag. × 8.
FIG. 14.—Multiple minute basal cell growths. Mag. × 64.

COMMENT ON GROUP C

These 4 early growths show: (a) a disproportionate ratio of superficial extent to depth; (b) clear evidence of multifocal origin from the basal layers of the epidermis or its derivatives. It appears to me to be beyond dispute that the superficial plaque-like basal cell carcinomas do not arise each from a single small focus and do not enlarge solely by proliferation, but that they show progressive neoplastic conversion of particular fields of epidermis and epidermal derivatives, often starting at multiple foci in those fields.

Case XIV plainly shows than an affected area may contain numerous minute, separate growths, and there is little doubt that with the lapse of time further intervening foci would continue to appear, and would eventually lead to coalescence of the entire group into what would then appear as a single plaque-like growth like those of Cases XI and XII. Even after the formation of such a composite plaque, additional independent foci of neoplastic change may continue to appear in the surrounding epidermis, as in Cases XII and XIII, and these may later coalesce with the main mass. Two distinct factors will contribute to coalescence of initially separate foci of growth; namely, neoplastic conversion of intervening epithelium and proliferative enlargement of adjacent cancerous masses.

These observations and conclusions are not original. The same conclusions were reached long ago by Lohmer (2), and more recently by Molesworth (3). The latter, in his admirable and thorough study of a large series of rodent carcinomas, insisted that "A rodent tumour may and frequently does arise from several and many points"; and again that "the main tumour mass is being reinforced continually by exactly similar cells produced not by reproduction from its own cells, but from those of neighbouring epithelium." Impressed by the clearly progressive origin of the growth in a field of epidermis, Molesworth envisaged "stimulation of the neighbouring epithelium into activity—by infecting it, as it were, with the fever which drove the original area of epithelium to proliferate." However, as I pointed out in my previous paper (4), we do not need to postulate any contagious influence passing from cell to cell; spreading neoplastic conversion of a field of tissue is merely the progressive response of an area of which all has been subjected to the same original stimuli; a response timed according to the gradients of the effective stimulation.

I have omitted description of examples of the more bulky subepidermal types of basal cell growth, such as the so-called "benign cystic epithelioma"; but these also may show clear signs of multifocal origin

from several groups of sweat glands, ducts, or pilosebaceous units. I am also satisfied that there is no real histogenetic difference between the superficial, plaque-like basal cell growths, of predominantly epidermal origin and prone to early ulceration, and the deeper bulkier growths, of predominantly glandular and follicular origin and not prone to ulceration. The basal cells in all these situations give rise to closely similar tumors; and a single tumor may show multiple origin from both epidermal and deeper epithelial structures, and may show the combined characters of the superficial rodent and the deeper "benign" cystic types of basal cell growth.

SURGICAL IMPLICATIONS

The specimens described in this and my earlier paper show that the mode of origin of epidermal tumors of the most diverse types is by progressive neoplastic change in fields of predisposed epithelium of variable size. It is, I think, very doubtful if an epidermal growth ever takes origin from a single minute focus at a single moment of time. The more closely early growths are studied, the more apparent does it become that transitions from normal to cancerous epithelium can be observed, and that cancerous change is still progressing.

The extent of the potentially cancerous field is an important question in practical surgery. As Hauser (1) said, "most surgeons will not accept Thiersch's view of regional recurrence, but assume that recurrence never takes place when the primary tumor has been completely extirpated." Growths like those of Group B above are the ones in which this surgical belief is liable to lead to inadequate operations. In most cases, carcinoma supervening on old burn scars or other extensive inflammatory lesions will probably recur if treated by purely local excision of developed areas of growth. The surgeon's notion that this has been due to incomplete removal of the tumor tissue already present is often erroneous. Removal has been inadequate, not as regards tumor tissue *per se*, but as regards incipiently cancerous tissue. Extensive extirpation of the entire predisposed field, amputation if need be, is the logically proper surgery for scars, ulcers, postradiation dermatitis, vulval leukoplakias, and other pre-existing lesions in which cancerous change has commenced. Even with some growths of quite localized origin postoperative recurrence is sometimes attributable to fresh cancerous change in the epithelium of the area, as in Case VI above.

There are some epidermal diseases with neoplastic potentialities so great and so widespread that the clinician recognizes the impracticability of radical surgery. The most striking of these is xeroderma pigmentosum, in which in early life the congenitally predisposed skin develops multiple cancers of both squamous cell and basal cell types; the only completely effective surgery in such a case would be flaying. To a less degree the same applies to those patients with highly sensitive skins who develop numerous tumors, such as Cases XIII and XIV above, and also those patients who display numerous subepidermal "benign" basal cell growths; surgery may remove some or many of the already established tumor foci, but cannot ablate the tumor-bearing field.

GENERAL CONCLUSIONS

1. Carcinomas of the skin of the most diverse types arise from fields of epithelium of greater or less extent. The areas of origin of the most circumscribed tumors are often 1 or 2 cm. in diameter. Carcinomas of scars and chronic ulcers and other precancerous inflammatory lesions may arise simultaneously or successively over extensive areas of epidermis.

2. Progressive cancerous change in areas of predisposed epithelium is often demonstrable in early tumors; it may commence at one focus or at multiple foci.

3. If a tumor is excised from a field of predisposed epidermis in which cancerous change is still in progress, recurrence of growth may take place by neoplastic conversion of residual parts of the field.

4. The still prevalent view that tumors usually arise each at a single point in time from a single small focus is false; it is but an echo of Cohnheim's and Ribbert's views, and should cease to influence modern pathologists.

ACKNOWLEDGMENT

I am indebted to Professor P. MacCallum for stimulating discussions; to members of the Surgical Staff of The Alfred Hospital for access to clinical records of their patients; to Lt. Colonel W. B. Wartman, of the U. S. Army Medical Corps, for specimen No. XIV; to Mr. R. Prosser for expert preparation of the microscopical sections; and to my wife for help with the photography.

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The Effect of 3,4-Benzpyrene on the Autoxidation of Unsaturated Fatty Acids*

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Previous publications from this laboratory have demonstrated that carcinogenic hydrocarbons inhibit the autoxidation of certain aldehydes and unsaturated lipids (3, 4, 11, 15), with a concurrent oxidative destruction of the hydrocarbon (9, 15). Since any information regarding a possible chemical activity of the hydrocarbon in a substance of biological importance may be pertinent to the problem of carcinogenesis, investigations on this subject have been continued. To obtain additional data about the influence of certain carcinogens on the oxidation of lipids, the effect of 3,4-benzpyrene on the autoxidation of purified unsaturated fatty acids was studied. The autoxidation of these lipids in the presence of different levels of benzpyrene was followed by determining the rate of oxygen consumption, the iodine number, and the peroxide value at regular intervals. The effect of this oxidation on the rate of benzpyrene destruction and benzpyrene quinone formation was also investigated.

METHODS

Uniform samples of purified linoleic or linolenic acid were prepared by transferring known quantities of these materials dissolved in Skelly solve B to Warburg flasks or test tubes; additions of 3,4-benzpyrene were made in the same manner. After all additions had been made the volatile solvent was removed in a vacuum desiccator at room temperature, leaving the lipid dispersed in thin droplets over the bottom of the flask. With this method accurate additions could be easily made and the results were much more uniform than when the fatty acids were pipetted directly into the flask. A typical test sample contained 5 mgm. of fatty acid and 10 to 160 μgm. of 3,4-benzpyrene. The flasks were connected to the manometers of a Warburg apparatus and the mixtures allowed to autoxidize for varying periods of time at 37° C. in the absence of light. After oxidation had proceeded for the desired period, the contents of the flasks were dissolved in 5 ml. of redistilled acetone or in peroxide-free ethyl ether, aliquots were transferred to appropriate containers, and the volatile solvent was removed in a vacuum desiccator at room temperature before further analyses were performed.

The iodine numbers were determined according to the Rosenmund-Kuhnhenn method (10), modified for 1 to 3 mgm. samples of the fatty acids. In a typical determination a 1 mgm. sample of fatty acid was dissolved in 5 ml. of chloroform and allowed to react with a 50 per cent excess of halogenating solution for 30 minutes. At the end of this period the excess bromine was titrated by the conventional iodometric procedure. The peroxide values were determined on 1 to 3 mgm. samples of fatty acid dissolved in 1 ml. of a 2:1 solution of glacial acetic acid and chloroform. About 100 mgm. of solid potassium iodide was then added to each tube and the mixture heated to 100° C. in a water bath for 2 minutes. The tubes were then rapidly cooled by placing them in a beaker of cold water and by adding 2 ml. of distilled water to each one. The iodine liberated by the peroxide was titrated from a microburette with approximately 0.0024 *N* sodium thiosulfate solution (standardized for each experiment with potassium dichromate solution). The 3,4-benzpyrene in the lipid mixtures was determined by fluorometric analysis according to the method described by Miller and Baumann (8).

Purified linoleic and linolenic acids were prepared by methods previously described (9). We are indebted to Dr. F. W. Quackenbush, formerly of the Department of Biochemistry, for these samples. Crystalline 3,4-benzpyrene, obtained from Hoffmann-LaRoche, Inc., was purified by adsorbing the benzpyrene from a solution in Skelly solve B on a column of activated alumina. The column was then pushed out of the glass tube and the section of alumina containing the adsorbed benzpyrene was eluted with acetone. The benzpyrene was recrystallized from the heated acetone solution by the addition of cold water. 3,4-Benzpyrene-5,8-quinone was prepared by oxidizing 3,4-benzpyrene with chromic acid (14), dissolving the oxidation products in benzene, and isolating the quinones by chromatographic adsorption on columns of activated alumina (9).

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

RESULTS

The autoxidation of linoleic acid followed the characteristic pattern of a slow initial period after which the rate increased and then finally decreased. A typical experiment in which the rate of autoxidation of 5 mgm. of linoleic acid was followed alone and with varying levels of carcinogen is presented in Fig. 1. Oxidation of the acid was retarded by the addition of 3,4-benzpyrene and the degree of inhibition was greater with large amounts of hydrocarbon than with small quantities, but the percentage inhibition was not directly proportional to the concentration of the carcinogen (Fig. 2). Thus during the period of most rapid oxidation (the interval from 30 to 40 hours) 90 per cent inhibition was obtained with 160 μgm. of benzpyrene as compared to 20 per cent inhibition obtained with 10 μgm. of carcinogen. When linolenic acid was allowed to autoxidize in the presence of varying amounts of benzpyrene the results were similar to those obtained with linoleic acid, but the rate of oxidation was considerably faster (Fig. 3); the inhibition obtained also was not directly proportional to the concentration of the hydrocarbon (Fig. 2).

At intervals during the autoxidation of the linoleic acid-benzpyrene mixtures the iodine number and peroxide value were determined. In the absence of benzpyrene the iodine number of oxidizing linoleic acid followed a typical pattern, comprising an initial period in which the decrease was gradual, a second stage of rapid change, and a third phase in which the rate was slow again (Fig. 4). The curve was the reverse of that observed for oxygen uptake (Fig. 1). When oxidation was retarded with benzpyrene the fall in iodine number was greatly delayed, and when higher levels of hydrocarbon were employed an increase in iodine number preceded the drop (Fig. 4). This increase in iodine number was confirmed on several occasions; calculations showed that only one-third of the observed increase could be accounted for

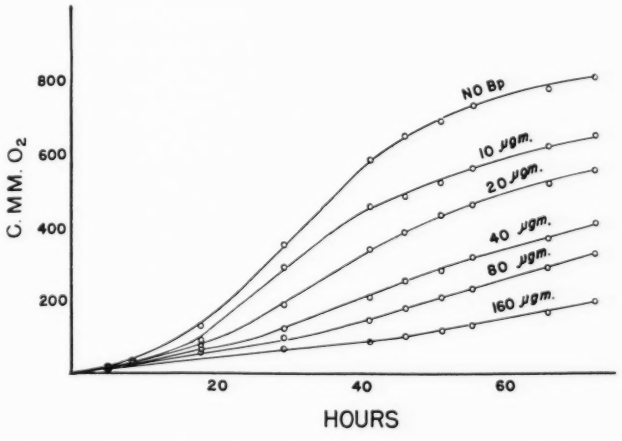

FIG. 1.—Inhibiting effect of various amounts of 3,4-benzpyrene on rate of autoxidation of linoleic acid.

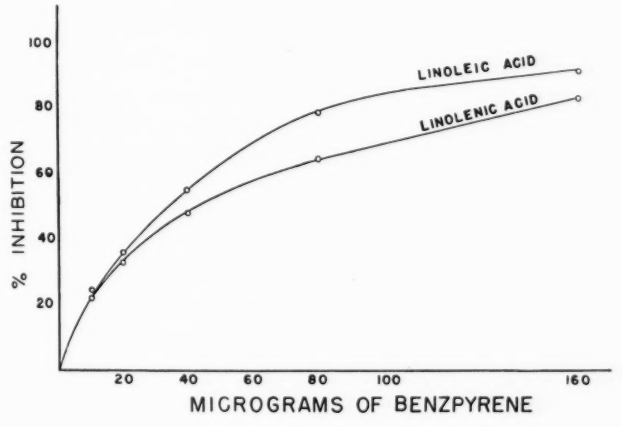

FIG. 2.—Percentage of inhibition of autoxidation of linoleic and linolenic acids induced by various amounts of 3,4-benzpyrene.

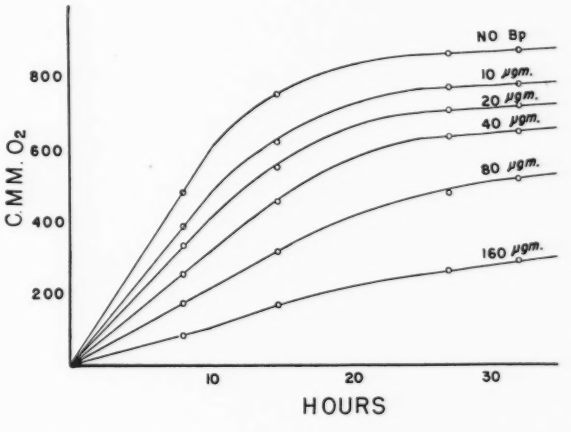

FIG. 3.—Inhibiting effect of various amounts of 3,4-benzpyrene on rate of autoxidation of linolenic acid.

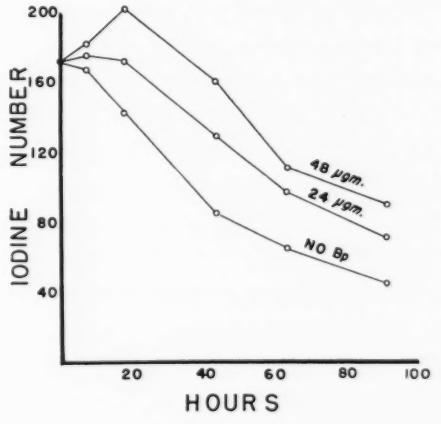

FIG. 4.—Effect of 24 and 48 μgm. of benzpyrene on autoxidation of linoleic acid as determined by change in iodine number.

if all the double bonds in the added benzpyrene had reacted in the determination of the iodine number. The cause of this effect is unknown, but it is possible that intermediate products related to the fatty acids accumulated during the inhibition of the oxidation of these acids with 3,4-benzpyrene. Such intermediates might be able to react with the halogenating solution, and when oxidation of the fatty acid is greatly retarded the net result would be a paradoxical rise in the iodine number. Although these interfering factors were presumably present throughout the period of oxidation, it is likely that their effect was eventually obscured by the mounting rate of oxygen uptake and the concomitant loss of fatty acid. The peroxide value of linoleic acid increased rapidly during autoxidation

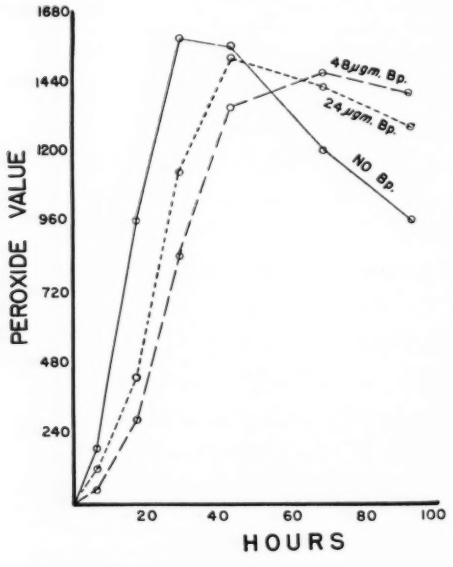

FIG. 5.—Effect of 24 and 48 μgm. of benzpyrene on autoxidation of linoleic acid as determined by change in peroxide value.

and reached a maximum at about 30 hours, after which there was a decline (Fig. 5). The addition of benzpyrene to the unsaturated fatty acid checked both the rate of increase and the secondary fall of the peroxide value. The changes in the iodine number and the peroxide value indicated the degree of autoxidation, and confirmed the results obtained by the measurement of the oxygen uptake.

In a previous investigation (9) it was shown that 3,4-benzpyrene was rapidly destroyed in oxidizing linoleic acid, and two colored oxidation products, 3,4-benzpyrene-5,8-quinone and 3,4-benzpyrene-5,10-quinone, were identified in the partially oxidized mixtures. In the present study the rate of autoxidation was compared to the degree of hydrocarbon disappearance and to the formation of the mixed quinones of benzpyrene (Fig. 6). Fig. 1 shows that autoxidation proceeded faster as the amount of benzpyrene in the mixture was decreased. When 43 μgm. of benzpyrene was added to 5 mgm. of linoleic acid a considerable decrease in the rate of autoxidation was observed, and the rate of hydrocarbon disappearance paralleled the degree of fatty acid oxidation; *i.e.*, it was slow in the beginning, then was more rapid, and finally became more gradual again. An exact correlation could not be made, however, since the destruction of benzpyrene was negligible during the first 20 hours of the experiment, even though the linoleic acid had undergone some autoxidation. It was also apparent that the percentage of benzpyrene destroyed was much greater when small amounts were added to the fatty acid, and the inhibition of autoxidation was considerably

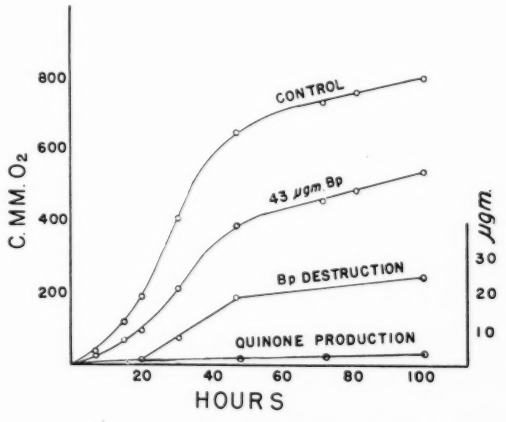

FIG. 6.—Rate of autoxidation of linoleic acid compared with degree of hydrocarbon disappearance and formation of mixed quinones of 3,4-benzpyrene. (The amount of oxygen uptake of linoleic acid alone or in the presence of benzpyrene is shown by the ordinate at the left and is indicated by the two top curves, while the ordinate at the right indicates the μgm. of benzpyrene destroyed and of the mixed quinones formed when 43 μgm. of the carcinogen was added to 5 mgm. of the fatty acid.)

less than when larger concentrations of the hydrocarbon were employed (Table I). When 10 μgm. of

TABLE I: THE DISAPPEARANCE OF 3,4-BENZPYRENE COMPARED WITH THE DEGREE OF INHIBITION OF LINOLEIC ACID AUTOXIDATION

The data presented in this table are from determinations made at the end of a 63 hour period of autoxidation.

Amt. of benzpyrene added, μgm.	Amt. of benzpyrene lost, μgm.	Loss of benzpyrene, per cent	Inhibition of autoxidation, per cent
10	9.2	92.0	19
20	15.0	75.0	31
40	19.5	49.0	48
80	27.2	34.0	59
160	13.5	8.5	75

benzpyrene was added to 5 mgm. of linoleic acid, autoxidation was inhibited by 19 per cent by the end of 63 hours and only 0.8 μgm. of hydrocarbon

remained in the mixture, but when 160 μgm. of carcinogen was employed 146.5 μgm. was detected at the end of the same period and the inhibition observed was 75 per cent. A gradual increase in the amount of the mixed quinones of benzpyrene was also observed during the autoxidation of linoleic acid, but the level of quinones detected at any one time accounted for only a small fraction of the destroyed hydrocarbon

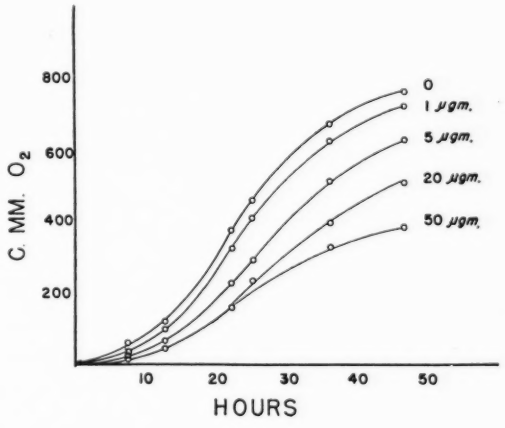

FIG. 7.—Inhibiting effect of various amounts of 3,4-benzpyrene-5,8-quinone on rate of autoxidation of linoleic acid.

(Fig. 6). This indicates that the quinones underwent further oxidative destruction.

These results seem to demonstrate that 3,4-benzpyrene inhibited the autoxidation of linoleic and linolenic acids from the outset (Figs. 1 and 3). This would indicate that the intact hydrocarbon *per se* was responsible for the initial inhibition. Further evidence to support this contention was gained from

TABLE II: THE DISAPPEARANCE OF VARIOUS LEVELS OF 3,4-BENZPYRENE-5,8-QUINONE COMPARED WITH THE DEGREE OF INHIBITION OF LINOLEIC ACID

The data presented in this table are from determinations made at the end of a 46 hour period of autoxidation.

Amt. of Bp-quinone added, μgm.	Amt. of Bp-quinone lost, μgm.	Loss of Bp-quinone, per cent	Inhibition of autoxidation, per cent
1	0.1	90	5
5	4.0	80	17
20	12.0	60	33
50	26.3	53	50

an experiment in which the hydrocarbon was added to linoleic acid 52 hours after the onset of oxidation. The addition of benzpyrene resulted in an immediate retardation of further autoxidation, and 20 hours after the carcinogen was added oxygen uptake was reduced by 57 per cent.

Although the results of the present experiments indicate that the intact benzpyrene molecule inhibits the autoxidation of unsaturated fatty acids, experiments were performed to determine whether any of the oxidized products of benzpyrene also possessed this property. When various levels of red 3,4-benzpyrene-5,8-quinone were added to 5 mgm. samples of linoleic acid, a significant inhibition of autoxidation was observed similar to that obtained with 3,4-benzpyrene itself (Fig. 7). In the first 24 hours there was no significant difference between the inhibition produced by 20 μgm. as compared to that produced by 50 μgm. of 3,4-benzpyrene-5,8-quinone but after this period a difference became evident and was pronounced at 46 hours. As autoxidation proceeded there was also a concomitant disappearance of the quinone (Table II). It is possible that still other substances resulting from the oxidation of benzpyrene likewise interfere with the autoxidation of fatty acids.

DISCUSSION

The observations made in this study indicate that the initial inhibition of fatty acid autoxidation is due to the intact benzpyrene molecule or to a primary reaction concerned with the destruction of the hydrocarbon. The fact that an oxidation product of 3,4-benzpyrene, *i.e.*, 3,4-benzpyrene-5,8-quinone, was also capable of retarding the autoxidation of linoleic acid suggests that other derivatives of benzpyrene may likewise possess this property. A destruction of 3,4-benzpyrene and of its quinone took place concurrently with the autoxidation of linoleic acid.

It has been suggested (9) that the destruction of benzpyrene in a mixture of autoxidizing fat might be the result of a coupled oxidation similar to that occurring when carotene (12, 13), *p*-dimethylaminoazobenzene (5), or hemin (7) are destroyed in the presence of oxidizing fats. The results of the present experiment strengthen the contention, since the rate of hydrocarbon disappearance parallels the speed of fatty acid autoxidation.

The relationship of the present findings to the problem of carcinogenesis is not clear. It is of interest, however, to note how readily a carcinogenic hydrocarbon can be destroyed *in vitro* in the presence of a substance that is also an important constituent of living cells. It is possible that polycyclic aromatic hydrocarbons undergo a similar oxidation *in vivo*, but there is no experimental evidence to support this suggestion. It is also of interest that 8-hydroxy-3,4-benzpyrene and 3,4-benzpyrene-5,8-quinone have been detected in the excreta of rats and mice that had been injected with 3,4-benzpyrene (1, 2). These derivatives are apparently noncarcinogenic (2). There are probably other ways in which carcinogenic hydrocarbons may be destroyed in the body, but the possibility of destruction as the result of oxidation is supported by

fact. At the present time there is uncertainty as to the chemical nature of the carcinogen that is directly responsible for the changes leading to cancer formation induced by carcinogenic hydrocarbons. Evidence thus far indicates that only the intact hydrocarbon molecule is carcinogenic (6), but only a few of the many possible products of metabolism have been tested as possible carcinogens. It is also probable that the *chemical reactions* involved in the degradation of a hydrocarbon *in vivo* lead to the changes necessary for neoplastic growth. If this suggestion were correct there would be no reason to expect that any derivative of such reaction possesses carcinogenic activity.

SUMMARY

The effect of 3,4-benzpyrene on the autoxidation of purified linoleic or linolenic acid at 37° C. was investigated by adding varying amounts of the hydrocarbon and determining the oxygen uptake, the iodine number, and the peroxide value of the mixtures at regular intervals. The rate of disappearance of the carcinogen from autoxidizing linoleic acid was also measured under similar conditions. The data demonstrate that the autoxidation of these acids was inhibited by all levels of benzpyrene employed, but large amounts of the hydrocarbon (3.2 per cent) were more effective than small amounts (0.2 per cent); however, the degree of inhibition was not directly proportional to the level of carcinogen employed. Benzpyrene was destroyed at a rate that was proportional to the speed of fatty acid oxidation. Only a small part of the carcinogen that had disappeared could be accounted for in the form of the mixed quinones of benzpyrene. 3,4-Benzpyrene-5,8-quinone also inhibited the autoxidation of linoleic acid and also was destroyed during the process.

The evidence indicates that 3,4-benzpyrene as well as the mixed quinones of this hydrocarbon undergo a coupled oxidation when dissolved in an autoxidizing unsaturated fatty acid.

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Testing Carcinogen Analogues for Carcinogenic Activity*

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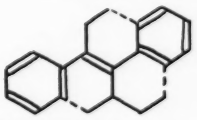
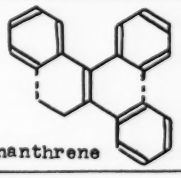
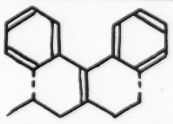
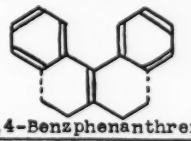
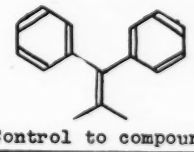
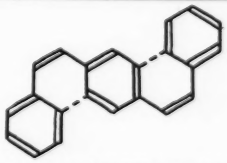
In 1941 we reported that *a*-ethyl-*β*-*sec*-butylstilbene (Lab. No. 616) had slight carcinogenic activity when painted on mice (1). The structural relations of this compound to benzpyrene or dimethylchrysene are similar to those between the synthetic and natural estrogens. We have since carried out tests on 4 other compounds that may be considered as analogues of active carcinogens in the same way that compound

* Because of the difficulties of international communication the authors have not read proof of this article.

TABLE I

Compound	Lab. no.	Skeleton formula and carcinogen to which compound is possibly related	No. of mice At start of exp.	No. of mice After 1 year	No. of tumors
α-Ethyl-β-sec-butyl-stilbene	616	 Benzpyrene	25 25 25 25	14 3 2 2	1 * 1 † 0 0
α:α:β-Triphenyl-α-butylene	772	 1,2,3,4-Dibenzphenanthrene	25 25	10 9	0 0
α:α-Diphenyl-β-ethyl-α-amylene	810	 2-Methyl-3,4-benzphenanthrene	25 25	4 2	0 0
α:α-Diphenyl-β-ethyl α-butylene	786	 3,4-Benzphenanthrene	25 25	5 3	0 0
α:α-Diphenyl-β-methyl α-propylene	851	 (Control to compound 786)	25 25	9 0	0 0
1,4-Distyryl-benzene	795	 1,2,5,6-Dibenzanthracene	25 25	15 0	0 0

* Spindle cell carcinoma.
† Spindle cell sarcoma.

616 is. These tests, and a repeat test of 616, gave negative results.

METHOD

Each compound was painted on the shoulders of groups of 25 adult male mice twice weekly for at least a year. The substances were all made up as 0.3 per cent solutions in benzene.

RESULTS

Details of the tests, and of the carcinogenic compounds to which the test substances are supposedly related, are given in the accompanying table.

DISCUSSION

The failure to produce any tumors in the second 50 mice painted with compound 616 does not invalidate the original observations, since its carcinogenic activity is admittedly slight and the number of mice surviving in the second test was smaller.

Dr. Haddow has kindly tested all these compounds for their ability to inhibit the growth of the Walker rat carcinosarcoma 256. In doses of 50 mgm. compounds 786, 795, and 851 were completely inactive, but the results with the other compounds were not so definite. Compounds 616 and 772 in 100 mgm. doses produced "extremely slight inhibition." Fifty milligrams of compound 810 produced "slight but definite inhibition." These results suggest that the compounds may have some carcinogenic activity but that it is too slight to be demonstrable by painting in groups of 50 mice. The mainly negative results are of course disappointing, but we do not think they are conclusive enough to disprove the theory underlying the experiment.

SUMMARY

Five compounds, whose structural relation to active carcinogens resembles that of stilbestrol to estrone, were tested for carcinogenic activity.

Slight activity was demonstrated in one of them—*α*-ethyl-*β*-*sec*-butylstilbene—which is regarded as a benzpyrene analogue.

ACKNOWLEDGMENT

We are very grateful to Dr. A. Haddow, of the Chester Beatty Research Institute, Royal Cancer Hospital (Free), for permission to quote his report on the activity of the compounds in inhibiting tumor growth.

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Abstracts

Reports of Research

On Tumor-Producing Chemical Substances. SHABAD, L. M. [A. M. Gorky All Union Inst. of Exper. Med., Moscow, Russia] *Cancer Research,* **5**:405-419. 1945.

A review of studies, made chiefly by the author and his associates during the past 8 years, on exogenous and endogenous tumor-producing substances. Among the exogenous agents investigated were 1,2,5,6-dibenzanthracene, 9,10-dimethyl-1,2-benzanthracene, 9-methyl-10-ethyl-1,2-benzanthracene, 3,4-benzpyrene, 3,4,8,9-dibenzpyrene and its 5,10-quinone, 9-methyl-1,2-benzanthryl-10-acetic acid, *o*-aminoazotoluene, *p*-dimethylaminoazobenzene, benzanthrene, tetramethyldiaminophenone, slate tars, industrial oils ("sulfofresol," "emulsol"), and a synthetic estrogen ("polianol") chemically simpler than folliculin. Data are summarized on the ability of these agents to produce tumors at or remote from the site of application, the transplantability of such tumors, the local effects of the agents (with special reference to inflammation), their antigenic action, the correlation between chemical structure and carcinogenesis, and the inhibition of experimental tumors by spleen extracts, spleen itself, and blood. The section on endogenous agents is devoted chiefly to the author's reports on the production of benign and malignant tumors in mice by injection of benzene extracts of the liver, bile, and lungs from persons dead of cancer of diverse type and location (occasionally in liver or lungs), and on the identification of the active agent with the non-saponifiable lipid fraction of such extracts. Theories of carcinogenesis are discussed. The bibliography contains 83 references.—M. H. P.

Bestimmung der Abnahme des 3,4-Benzpyrens aus Paraffindepots bei Mäusen. [Determination of the Decrease of 3,4-Benzpyrene in Paraffin Depots in Mice.] ROSENBOHM, L., and ROSENBAUM, A. [Univ.-Krankenhaus, Hamburg-Eppendorf, Germany] *Ztschr. f. Krebsforsch.,* **53**:254-262. 1943. From abstr. in *Chem. Zentralbl.,* **1**:2098. 1943.

Paraffin depots containing various amounts of benzpyrene were placed in 558 mice of pure strain, and removed after various intervals for determination of the remaining benzpyrene by a method involving fluorescence. Ten days after the subcutaneous implantation of 1,333γ, of 200 to 266γ, and of 133γ of the carcinogen, the percentages remaining in the respective loci were 15, 10, and 4.7. After intraperitoneal injection of the same amounts, 8.2, 12.0, and 3.5%, respectively, were found. Abdominal tumors occurred in 10 of 29 animals that died 100 to 240 days after intraperitoneal injection. After the amount in the depot dropped from 1,333γ to 200γ, the level fell to 3γ in 130 days, though when the original amount was 200γ, the level of 3γ was reached in only 30 days. Thus with the high initial amount of 1,333γ there occurred an inhibition of continued diffusion, attributed to a preliminary uptake by the surrounding tissue. The incidence of tumors appears to be great if a large amount of implanted benzpyrene diffuses first rapidly, then slowly, from the depot.—M. H. P.

Metabolism of 3,4-Benzpyrene in Mice. WEIGERT, F. [Mt. Vernon Hosp., Northwood, Middlesex, England] *Nature, London,* **155**:479-480. 1945.

The metabolism of 3,4-benzpyrene in the mouse was studied with the aid of chromatographic adsorption analysis and absorption spectrography. It is suggested that the hydrocarbon passes through 4 intermediate stages. In the first 2 stages, 1 of the rings is reduced, and the nucleus is therefore no longer completely aromatic (*e.g.* 8,9-dihydroxy-8,9-dihydro-3,4-benzpyrene). In stages 3 and 4 the aromatic nucleus is restored, this fourth stage being represented by 8-hydroxy-3,4-benzpyrene.—I. H.

The Direct Hydroxylation of 1′:2′:3′:4′-Tetrahydro-3:4-benzpyrene. KON, G. A. R., and ROE, E. M. F. [Chester Beatty Research Inst., Roy. Cancer Hosp. (Free), London, England] *J. Chem. Soc.,* 143-146. 1945.

The synthesis of 8-hydroxy-3,4-benzpyrene, which appears to be a metabolic product of the carcinogenic hydrocarbon 3,4-benzpyrene, was attempted by hydroxylation of 1′,2′,3′,4′-tetrahydro-3,4-benzpyrene by means of lead acetate. But the saturated ring was attacked almost exclusively, giving the 1′-acetoxy derivative, and a hydrocarbon that appeared to be 3′,4′-dihydro-3,4-benzpyrene.—E. L. K.

The Disappearance of Carcinogenic Hydrocarbons in Autoxidizing Lipids. MUELLER, G. C., MILLER, J. A., and RUSCH, H. P. [Med. Sch., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **5**:401-404. 1945.

The rates of destruction of 3,4-benzpyrene, 20-methylcholanthrene, and 1,2,5,6-dibenzanthracene were followed fluorometrically after these hydrocarbons were added singly to various lipids and the mixtures exposed to air at room temperature for several weeks. Benzpyrene and methylcholanthrene were rapidly destroyed in oxidizing linoleic acid, and losses of 34 and 84% respectively were noted after 1 week. 1,2,5,6-Dibenzanthracene was relatively stable to this oxidizing agent. Benzpyrene also disappeared, though less rapidly, in ethyl linolate, ethyl oleate, and chromatographed cottonseed oil. The addition of tocopherol to certain of these mixtures delayed the onset of the destruction but did not alter the final result. Benzpyrene was relatively stable when dissolved in tricaprylin, lard fractions, or mouse carcass fat.

The isolation of 2 of the colored oxidation products of benzpyrene was accomplished by chromatographic adsorption of an autoxidized benzpyrene-linoleic acid

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mixture on activated alumina. These compounds were identified as 3,4-benzpyrene-5,8-quinone and 3,4-benzpyrene-5,10-quinone by mixed chromatographs with the authentic quinones and by their absorption spectra.—Authors' abstract.

The Recovery of Carcinogenic Hydrocarbons from Solution by the Use of Picric Acid. Jones, R. N., and Jamieson, J. R. [Queen's Univ., Kingston, Canada] *Cancer Research,* **5**:341-345. 1945.

Dibenzanthracene, benzpyrene, and methylcholanthrene, 0.5 to 5.0 mgm., can be removed from ethanolic solution by conversion to picric acid complexes. The recovery yields, estimated by ultraviolet spectrophotometry, were 70 to 90% for dibenzanthracene, 30 to 60% for benzpyrene, and 20 to 40% for methylcholanthrene. Satisfactory recoveries have been obtained in the presence of large excesses of stanolax, a neutral mineral oil; from this it may be inferred that the technic is applicable to the extraction of small amounts of carcinogenic hydrocarbons from neutral oils of biological origin.

Fluorescent neutral oils can be obtained from the nonsaponifiable fractions of human livers after removal of sterol by crystallization, chromatographic adsorption, and high vacuum distillation. These concentrates when treated with picric acid in the absence of stanolax did not form picric acid complexes; the "adsorbates" reported by Jones and May (*Cancer Research,* **4**:303. 1944; abstr. in *ibid.,* **4**:389. 1944) are now believed to have been mechanical mixtures.—Authors' abstract.

Healing of Wounds in the Skin of Mice Painted with 20-Methylcholanthrene. Silberberg, M., and Silberberg, R. [Jewish Hosp., and St. Louis City Hosp., St. Louis, Mo.] *Arch. Path.,* **39**:257-264. 1945.

Methylcholanthrene applied to the epidermis of mice before the making of a wound influences the course of regeneration in a way similar to benzpyrene. The application of methylcholanthrene for 2 weeks or for 1 month intensifies proliferation and migration of the epithelial cells, and thus hastens the healing of the wound. But after 3 months of treatment with methylcholanthrene, cell migration is markedly inhibited and wound healing delayed in spite of the greatly increased proliferation of the epidermal cells. Between these two phases there is an intermediate stage in which the effect of methylcholanthrene differs from that of benzpyrene so far as the inhibition of cell migration is only transitory in the case of methylcholanthrene and is subsequently compensated for by rapid epithelization of the wound. The effect of methylcholanthrene on healing of wounds is thus a more quantitatively graded one than that of benzpyrene.—Authors' summary. (J. G. K.)

Tissue Nucleic Acids. 3. The Nucleic Acid and Nucleotide Content of Liver Tissue. Davidson, J. N., and Waymouth, C. [Univ. of Aberdeen, Aberdeen, Scotland] *Biochem. J.,* **38**:379-385. 1944.

The content of nucleoprotein, nucleotide, and nucleoside was determined: in the liver of normal male and female rats (a); in the liver of fasting, pregnant, and embryo rats; in regenerating liver; in liver tumors induced in 100 or 150 days by food containing dimethylaminoazobenzene in arachis oil (b); and in the liver of control rats receiving arachis oil only (c). The water content was higher in (b) than in (a). Nucleoprotein phosphorus in dry liver was higher in (b) than in (c) and higher in (c) than in (a). "In the 100-day tumours the nucleotide concentration is significantly lower on a wet-weight, but not on a dry-weight, basis than that of the controls. No appreciable variations in the nucleoside + free purine fraction were found." About 80% of the nucleic acid in (a) and (c) was ribonucleic acid. The proportion of desoxyribonucleic acid was higher in (b) than in (a).—E. L. K.

Tissue Changes in Experimental Mice Treated with Pentose Nucleotides. Parsons, L. D. [London Hosp., London, England] *J. Path. & Bact.,* **57**:9-20. 1945.

Large ring-like deposits of amyloid appeared around the Malpighian bodies of the spleen in:

(a) mice receiving injections of a commercial preparation, given clinically for agranulocytosis, of the sodium salts of ribonucleotides (8% watery solution with 0.3% cresol) from yeast (referred to below as "nucleotide"); the change was seen after from 17 to 100 days (5 injections weekly); and

(b) CBA mice bearing grafted generations of sarcoma induced in this strain by methylcholanthrene; the change occurred in 93% of the mice grafted.

Amyloid change was not found in normal or in irradiated mice, or, before the development of the tumor, in mice (stock or CBA) receiving carcinogenic compounds (1,2,5,6-dibenzanthracene-9,10-endo-αβ-succinic acid or methylcholanthrene).

In (a), no tumors were induced. Leukocytosis (up to 62,000) of both polymorphs and lymphocytes occurred, and the former sometimes outnumbered the latter. Lymph nodes showed increase of reticulum cells, of plasma cells, and of myeloid cells; giant cells occasionally appeared. Amyloid change occurred in the liver also. The spleen was of less than normal size. Nucleotide injection did not accelerate tumor induction by 1,2,5,6-dibenzanthracene-9,10-endo-αβ-succinic acid.

In (b), simultaneous treatment with nucleotide increased the growth of the grafts and greatly lessened the resistance of old CBA mice to these grafts. In grafted mice, nucleotide injection may lessen the incidence of amyloid change; this anomaly is under investigation. The high leukocytosis usually present in the blood of the grafted mice did not seem to be affected by treatment with nucleotides.

The similarity of the amyloid changes in (a) and (b) suggests that in (b) nucleotides may be liberated from the tumor. The investigation is to be extended with the use of pure nucleotides prepared by Professor J. M. Gulland. In an addendum Professor H. M. Turnbull describes the staining reactions which show that the substance in question is mouse amyloid.—E. L. K.

The Influence of Diet on the Production of Tumors of the Liver by Butter Yellow. Opie, E. L. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.,* **80**:219-230. 1944.

It was found that the frequency of the production of tumors by *p*-dimethylaminoazobenzene was directly re-

lated to the amount of fat and the presence of rice in the diet, and to the production of liver cirrhosis by the diet. The presence of fat in the diet hastened the appearance of tumors, and the substitution of rice for sugar in the diet increased the frequency of tumor production. Also it was observed that although *p*-dimethylaminoazobenzene produced tumors in the absence of liver cirrhosis, the frequency of tumors increased with the severity of cirrhosis. The agency by which rice favors the production of hepatic tumors is not yet known.—D. S.

The Influence of Caloric Restriction and of Dietary Fat on Tumor Formation with Ultraviolet Radiation. Rusch, H. P., Kline, B. E., and Baumann, C. A. [Med. Sch., and Coll. of Agric., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **5**:431-435. 1945.

The influence of calories on the development of tumors due to ultraviolet irradiation was determined in strain C albino mice, and the effect of the fat in the diet and its relation to the caloric content was investigated at the same time. The amount of ultraviolet irradiation received by the mice was approximately 3.6×10^7 ergs/cm^2 daily. The animals were divided into 4 groups of 48 each. Two groups received a calculated 6.7 calories per mouse per day, and the other 2 an average of 9.7. One group on the high, and one on the low calorie level were given a low amount of fat in the diet, whereas the 2 other groups were given a moderate amount of fat. At the end of 9 months the incidence of ear tumors in the 4 groups of mice was as follows: 87% in the high calorie-low fat group, 63% in the high calorie-moderate fat group, 24% in the low calorie-moderate fat group, and 7% in the low calorie-low fat group.

The data of this experiment and of others previously recorded suggest that most of the accelerating action of fat on tumor formation can be explained on the basis of an increased caloric intake, but that fat *per se* also appears to increase the rate of tumor formation. The latter effect is particularly evident when the total intake of calories is restricted.—Authors' abstract.

Studies on Cancer. I. The Relationship of Function, Light and Temperature to Growth by Mitosis. Blumenfeld, C. M. [West. Reserve Univ., and Univ. Hosps., Cleveland, Ohio] *Arch. Path.,* **38**:321-325. 1944.

Numerous observations made on 7 litters of Wistar rats indicated that in the young animals there was regularly a diurnal variation of the volume of urine excreted, with the rate of mitosis in the renal cortex varying inversely with this volume. The same animals likewise showed regularly a diurnal variation in the weight of food eaten, and the rate of mitosis in the submaxillary glands varied inversely with this. On the other hand, variations in light and dark for a period of 2 days and small changes in temperature of body or room have no effect on, or relationship to, the diurnal periodicity of mitotic activity in the epidermis of the albino rat. The data seemed to the author to provide a factual basis for the view that function restrains growth.—J. G. K.

Experimental Roentgen Injury. V. Effects on Hematopoietic Reserves and Regenerative Capacity. Henshaw, P. S., Thompson, J. W., and Meyer, H. L. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**:233-247. 1945.

The additive effects of x-rays in doses ranging from 12.5 to 400 r were studied in C3H male mice. It was found that the number of treatments required to bring the blood lymphocyte level below 4,000 per cu. mm. became less, and the period of recovery became longer, as the experiment progressed. Eventually all the animals developed a persisting lymphopenia, and irrespective of the size of the treatment doses this persisting lymphopenia occurred when the accumulated doses reached 300 to 500 r. These results were interpreted as indicating that the effects of subliminal doses of x-ray are additive, that destruction of lymphocytes occurs with each small dose, that the rate of this destruction must exceed that of lymphocyte production, and that the persisting lymphopenia marks the beginning of lymphoid reserve exhaustion. The remarkable ability of hematopoietic tissue, especially bone marrow, to regenerate was also discussed.—R. A. H.

Effect of X-Rays on the Transmissibility of Fowl Sarcoma in Its Nonfilterable Phase. Miszurski, B., Pikovski, M., Goldhaber, G., and Doljanski, L. [Hebrew Univ., Jerusalem, Palestine] *Cancer Research,* **5**:422-425. 1945.

Five out of 10 slowly growing Rous fowl sarcomas were found to be nontransmissible by both Berkefeld and paper-pulp-sand filtrates. After irradiation with doses lethal for the sarcoma cells, but practically harmless to the causative agent, 3 of these 5 tumors could be transmitted, while the remaining 2 could not. The significance of these findings for the understanding of the so-called nonfilterable phase of the fowl tumors is discussed.—Authors' summary.

Methylcholanthrene Papillomas and the Virus Problem. Woglom, W. H. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research,* **5**:420-421. 1945.

Of 131 mice inoculated with extracts of methylcholanthrene papillomas, not a single one developed a tumor. —Author's summary.

The Effect of Progesterone and Testosterone Propionate on the Growth of Mammary Cancer in Mice. Heiman, J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research,* **5**:426-430. 1945.

Subcutaneous injections of testosterone propionate and progesterone were given for some 6 months to female mice of the RIII strain. The treated animals were between 2 and 6 months of age. During this age period spontaneous mammary adenocarcinoma rarely appears in this strain. The incidence of these tumors in untreated animals between 6 and 12 months of age ranges between 54 and 90%. This incidence is reduced to 16.6% after progesterone and to 6.2% after progesterone combined with testosterone propionate. Ninety-six injected mice were observed until death or for more than 1 year. Eighteen animals, similarly treated and then inoculated with carcinoma or sarcoma, grew the transplanted tumor. Testosterone propionate and progesterone do not inhibit malignant growth after it is established. These hormones probably initiate involutionary changes in the normal

mammary gland of young females, which interfere with the early stages of abnormal growth.—Author's abstract.

Some Endocrinologic Considerations of Canine Neoplastic Diseases. MULLIGAN, R. M. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Arch. Path.*, **39**:162-171. 1945.

A review of the literature on canine oncology revealed several leads that might be followed in future investigation of neoplasms of the endocrine glands and of the genital tracts of both sexes. These included a syndrome of feminization in male dogs associated with testicular carcinoma, the relation between benign prostatic hypertrophy and castration, the coexistence of changes in the female genital tract with cysts and solid tumors of the ovary, the presence of hyperinsulinism with neoplasms of the pancreatic islets, the problem of the interpretation of proliferation of interstitial cells of the testes, the paucity of neoplasms reported for the pituitary, parathyroid, thymus and adrenal glands, the possible etiologic connection of hormones with venereal sarcoma, and the embryologic and hormonologic aspects of mammary tumors.—Author's summary. (J. G. K.)

The Cytology of the Contagious (Venereal) Tumour of the Dog. JACKSON, C. [Onderstepoort, Union of South Africa] *Onderstepoort J. Vet. Sc. & Animal Indust.*, **20**:97-118. 1944.

From a cytological study of the contagious venereal tumor of dogs, it is shown that the tumor cells are exceedingly rich in lipid globules, a feature that has previously been overlooked because the globules are dissolved out from paraffin sections, and because in frozen sections they are refractory to the customary methods for demonstration of fatty substances. To stain these globules, a specially devised acetic-carbol-sudan method is recommended. Current views—that the tumor is composed of round cells or of reticulum cells—the author believes to depend entirely on post-fixation artefacts. From present conceptions of the structure of large lymphocytes (lymphoblasts), he finds it difficult to identify the tumor cells with these elements, and suggests that the present results afford support for his alternative theory, that the contagious venereal tumor may be an apolar neuroblastoma. —A. H.

Anti-Reticular Cytotoxic Serum as a Means of Pathogenetic Therapy. BOGOMOLETS, A. A. *Am. Rev. Soviet Med.*, **1**:101-112. 1943.

The treatment of infections, fractures, diseases of the nervous system, and cancer is described.

The cellular elements of the connective tissue have been found by the author and his co-workers to increase the resistance of the body to cancer: the fibroblasts and microphages form a strong line of demarcation around cancer foci, hinder their infiltration of the adjacent tissues, and by growing into the tumors help replace cancer cells with benign tissue, while the macrophages seem to destroy the carcinogenetic cellular elements. These protective reactions are stimulated by small doses, inhibited by large doses, of anti-reticular cytotoxic serum (ACS).

ACS is produced for human therapeutic (subcutaneous) use by inoculating horses with the cells of the spleen and bone marrow from persons who died suddenly without infectious disease. Its use is reported to have considerably diminished the recurrence rate after operations for gastric and thoracic cancer. In inoperable cancer, ACS has improved the general condition of the patients, sometimes prolonged life, abolished or reduced pain, and led to the disappearance of metastases.

In experiments upon tumor transplantation in animals, although large doses of ACS favored the progressive development of the transplants, small doses greatly reduced the number of takes. A continuation of the experiments revealed the complete disappearance of large "carcinogenous tumors," and a decrease in the number of lung metastases, in mice receiving the serum.

This is a general article without presentation of original data for evaluation.—M. H. P.

A Method of Preparing and Preserving Anti-Reticular Cytotoxic Serum. MARCHUK, P. D. *J. méd. de l'Acad. d. Sc. de la RSS d'Ukraine*, **9**:1175-1189. 1939; *Am. Rev. Soviet Med.*, **1**:113-123. 1943.

Details are given of the preparation, assay, preservation, properties, and dosage of anti-reticular cytotoxic serum for use in investigations of cancer and other diseases in man and the lower animals.—M. H. P.

Anti-Reticular Immune Serum: Its Action Demonstrated by Tissue Culture Technique. POMERAT, C. M., and ANIGSTEIN, L. [Med. Branch, Univ. of Texas, Galveston, Texas] *Science*, **100**:456. 1944.

The inhibiting action of anti-reticular cytotoxic serum (ACS) on the growth of certain tissues, reported by Bogomolets (*Am. Rev. Soviet Med.*, **1**:101. 1943; abstr. appears earlier on this page), has been studied in experiments *in vitro*. The ACS used was prepared by immunizing rabbits against spleen and rib marrow of guinea pigs. It inhibited the cellular outgrowth of adult guinea pig spleen in tissue culture, but not that of chick embryo spleen.—M. H. P.

Some Pharmacological and Biological Effects of the Latex of Ficus Carica L. ULLMAN, S. B., HALBERSTAEDTER, L., and LEIBOWITZ, J. [Hebrew Univ., Jerusalem, Palestine] *Exper. Med. & Surg.*, **3**:11-23. 1945.

Latex from the fig tree, *Ficus carica L.*, given subcutaneously, inhibited the growth of subcutaneously transplanted benzpyrene sarcomas B, 616, and 2192 in rats. An alkaloid fraction (A) of the latex, and a nondialyzable, nonprotein, nonalkaloid fraction (C), injected subcutaneously into rats, rendered them practically nonsusceptible to subsequent subcutaneous transplantation of benzpyrene sarcoma 616. The same fractions, given subcutaneously and intravenously, produced regression of 30 to 50% of intraperitoneal and subcutaneous sarcoma transplants. The unfractionated latex and a globulin fraction had a strongly necrotic action on the skin, and when injected intravenously were very toxic. An alcohol-soluble fraction produced anemia. The susceptibility of benzpyrene sarcoma 616 to the therapeutic action of x-rays, which normally is very low, was increased considerably by subcutaneous injection of fractions A and C.—M. H. P.

Progress in Cancer Research. MORTON, J. J. [Univ. of Rochester Sch. of Med. and Dent., Rochester, N. Y.] *Connecticut M. J.*, **9**:167-177. 1945.

The main advances in cancer research are discussed under the headings: new experimental material; tissue

changes in carcinogenesis; hormonal, enzymatic, and tissue culture studies; effects of light; heterotransplantation; viruses; and studies on human cancer. To the author, the most significant investigations of the last 5 years have been in the production of malignancy *in vitro;* the production of carcinoma in animals from their own intrinsic chemical factors; the hormonal control of some cancers; the work upon heterotransplantation; the modification of viruses by passage in young and alien hosts; additional discoveries on the milk factor; and the studies on the effects of light. A bibliography of 124 references is included.—M. E. H.

The Problems of Cancer Biology. Spencer, R. R. [Nat. Cancer Inst., Bethesda, Md.] *J. A. M. A.,* **127**:509-514. 1945.

A general article reviewing many phases of the cancer problem and outlining the present status of cancer control and research.—M. E. H.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Heredity

Neuroblastoma of the Adrenal Medulla in Siblings. Dodge, H. J., and Benner, M. C. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Rocky Mountain M. J.,* **42**:35-38. 1945.

A report of neuroblastoma of the right adrenal in a brother and sister.—M. E. H.

Diagnosis—General

Significance of Supraclavicular Signal Node in Patients with Abdominal and Thoracic Cancer. A Study of One Hundred and Twenty-Two Cases. Viacava, E. P., and Pack, G. T. [Memorial Hosp., New York, N. Y.] *Arch. Surg.,* **48**:109-119. 1944.

Among 4,365 patients treated for thoracic or abdominal cancers, 122 (2.8%) had metastases to supraclavicular lymph nodes as evidenced by microscopic examination. Of these 122 patients, 73 had involvement of the signal node on the left side, 31 on the right side, and 18 on both sides. Involvement of the right side and bilateral involvement were more frequent in patients with tumors of the thoracic cavity. In 41 patients the supraclavicular metastases were the first indication of a malignant tumor. The percentage of supraclavicular metastases associated with cancer of different viscera varied: lung, 13.2% of 334 cases; esophagus, 7.1% of 210 cases; stomach, 2.6% of 883 cases; ovary, 6.1% of 148 cases; cervix uteri, 1.5% of 336 cases; testicle, 4.8% of 166 cases; and none among 54 cancers of the small intestine, 17 of the gall bladder, and 208 of the urinary bladder. Radium pack or deep x-ray therapy may relieve local symptoms, but the length of life is not appreciably increased.—W. A. B.

Radiation

The Use of Radon Seeds in the Treatment of Neoplasms. Kaplan, I. I. [Bellevue Hosp., and New York Univ. Coll. of Med., New York, N. Y.] *Urol. & Cutan. Rev.,* **48**:122-124. 1944.

A brief review.—V. F. M.

Adequate X-Ray and Radium Dosage. Ernst, E. C. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America,* **24**:1003-1021. 1944.

An illustrated lecture on: biological radiation effects; roentgen unit research developments; the double cross-arm vaginal radium applicator; radium distribution in cancer of the cervix; dosage records; comparison of effects of x-ray and radium; and radiotherapy of cancer of the skin, breast, larynx, pharynx, tonsils, and cervix.—J. L. M.

The Evolution of an Improved Transvaginal Speculum. Erskine, A. W. [Cedar Rapids, Iowa] *Radiology,* **43**:170-174. 1944.

A description of a new instrument for use in x-ray therapy of cancer of the cervix.—R. E. S.

Carcinoma of the Bladder: An Improved Technique for the Cystoscopic Implantation of Radium Element. Moore, T. D. [Memphis, Tenn.] *J. Urol.,* **51**:496-504. 1944.

The author describes a method for cystoscopic implantation of radium that permits removal after an allotted time, and he presents histories of 4 cases in which the method was used.—V. F. M.

The Treatment of an Unusual Hemangioma. Kaplan, I. I. [Bellevue Hosp., and New York Univ. Coll. of Med., New York, N. Y.] *Urol. & Cutan. Rev.,* **48**:290-291. 1944.

The case of a Negro with a huge hemangioma of the lip treated with radium needles is presented, with photographs.—V. F. M.

Roentgen Diagnosis of Bronchiogenic Carcinoma. Shinall, H. L. [St. Louis City Hosp., St. Louis, Mo.] *Radiology,* **42**:213-219. 1944.

From an analysis of 40 cases of bronchogenic carcinoma in which roentgenographic, clinical, and postmortem studies were made, the author concludes that a diagnosis can be made in the large majority of uncomplicated cases by the finding of a rounded nodular shadow, usually in the hilar region, when this finding is correlated with clinical history.—R. E. S.

Lung Abscess Secondary to Stenosing Bronchiogenic Carcinoma. Kraft, E. [City Hosp., New York, N. Y.] *Radiology,* **43**:39-47. 1944.

Three cases of bronchogenic carcinoma with secondary lung abscess are reported, with roentgenograms.—R. E. S.

The Roentgenologic Features of Mediastinal Tumors. Robbins, L. L. [Massachusetts Gen. Hosp., Boston, Mass.] *Radiology,* **43**:115-121. 1944.

The various mediastinal tumors cannot be exactly differentiated by roentgenological appearance, but certain diagnostic features have been demonstrated.—R. E. S.

Diagnosis and Treatment of Osteoclastoma. Brailsford, J. F. [Birmingham, England] *Radiology,* **43**:35-38. 1944.

A discussion of the diagnosis and treatment of osteoclastoma is presented. Biopsy and surgery are not recommended, the author depending on radiographic appearance for diagnosis and on roentgen therapy for treatment.—R. E. S.

Hypertrophic Gastritis Simulating Intramural Tumor of the Stomach. Hinkel, C. L. [Walter Reed Gen. Hosp., Washington, D. C.] *Am. J. Roentgenol.,* **53**:20-27. 1945.

A case report with review and discussion. Inflammatory diseases of the stomach are not infrequently mistaken for tumors. Differential diagnosis is sometimes difficult even for the roentgenologist and the gastroscopist. Chronicity, slight bleeding, vomiting, and achlorhydria suggest gastritis, with or without polypoid or adenomatous changes. The treatment is surgical because of the high incidence of hemorrhage and malignant change.—E. H. Q.

Roentgen Diagnosis of Incipient Cancer of the Rectum. Oppenheimer, A. [Laconia Hosp., Laconia, N. H.] *Am. J. Roentgenol.,* **52**:637-646. 1944.

Cancer of the rectum usually remains clinically silent until it has become quite large; in early stages it is usually discovered only during a routine examination. Therefore any routine study of the digestive tract should include careful examination of the rectum by the use of an opaque enema. A technic is described that provides accurate control of the distribution of the opaque medium in the rectum and sigmoid. The most constant sign of incipient cancer of the rectum is a small ulcer with rigid walls. Malignant polyps and small annular cancers not noted by digital examination may often be found roentgenologically. Suppurative pericolitis may give roentgen appearances simulating cancer, and care should be used in making the differential diagnosis.—E. H. Q.

Breast

Malignant Tumors of the Male Breast. Peck, M. E. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1108-1125. 1944.

During the past 30 years, among 65,000 patients with a clinical diagnosis of neoplastic disease, seen at the Barnard Free Skin and Cancer Hospital, 2,500 breast tumors were observed, of which 1,722 were verified by pathological study. Of the verified breast tumors, 43 were in males; of these, 15 were cancer.

"Radical operation in operable cancer [of the male breast] is the only treatment offering any hope for cure. . . . Criteria of operability are set down. Radiation therapy is an adjunct to radical operation, but it is indicated routinely only in cases having proved axillary metastases at operation or in cases of inoperable carcinoma. An analysis of the end results in this series reveals 5 year cures in only 20%. The literature dealing with carcinoma of the male breast supports this very disappointing and challenging result. It is suggested that earlier recognition of all breast tumors by the local doctor, with advocation of immediate operative investigation and radical therapy in the event cancer is found, might improve the general outcome. The 2 cases of fibrosarcoma of the male breast in the series are briefly discussed. Simple mammectomy is advocated as the treatment of choice for sarcoma of the male breast."—J. L. M.

Metastatic Carcinoma of the Breast. Scaricaciottoli, T. M. [Jefferson Med. Coll., Philadelphia, Pa.] *Arch. Path.,* **38**:337-338. 1944.

Report of a case in which a primary bronchogenic carcinoma metastasized to the breast.—J. G. K.

Ganglioneuroma of the Breast in a Five Year Old Child. Lages Netto, D. J. [Rio de Janeiro, Brazil] *Am. J. Dis. Child.,* **67**:382-383. 1944.

A case report.—R. A. H.

Male Genital Tract

Surgical Removal of Cancer of the Prostate Gland. The Radical Operation. Colston, J. A. C. [Johns Hopkins Hosp., Baltimore, Md.] *J. A. M. A.,* **127**:69-72. 1945.

The disease in its earliest stages is readily amenable to complete surgical eradication. The importance of early diagnosis, as in all other types of malignant disease, is stressed.—M. E. H.

Orchidectomy for Carcinoma of the Prostate: Personal Experiences. Rathbun, N. P. [Brooklyn, N. Y.] *J. Urol.,* **52**:326-329. 1944.

A discussion of personal experience, based on 23 castrations for prostatic cancer. The operation was performed as an adjunct to other procedures in 21 of the patients, and as the sole form of treatment in 2. Although 19 patients, including the last-mentioned 2, showed various degrees of improvement for periods ranging from 4 months to 3 years, the author expresses no hope that orchidectomy will ever cure.—V. F. M.

Prostatic Carcinoma Treated by Orchidectomy—Testicular Prosthesis. Tolson, H. L. [Cumberland, Md.] *Urol. & Cutan. Rev.,* **48**:130. 1944.

Of 28 patients subjected during 1941 to 1944 to orchidectomy for advanced prostatic carcinoma, 17 were comfortable and active at the time of writing (2 months to over 2 years after operation), 2 showed progression of the carcinoma, and 9 had died. Twelve of 20 patients having pain other than bladder pain were relieved of discomfort at least temporarily by orchidectomy. Nine patients had a marked increase in weight. A testicular prosthesis of plastic is advocated.—V. F. M.

Prostatic Carcinoma: A Subsequent Report. Marquardt, C. R. [Marquette Univ. Sch. of Med., Milwaukee, Wis.] *Urol. & Cutan. Rev.,* **48**:168-169. 1944.

The mortality in a group of 33 patients with inoperable prostatic cancer treated by castration or stilbestrol was 21.2% at the end of 1 year (*Ibid.,* **46**:343. 1942; abstr. in *Cancer Research,* **3**:795. 1943). The mortality of the same group at the end of 3 years was 81.8%. Of the 6 surviving patients, 4 were castrates and 2 were under stilbestrol treatment; only the latter 2 were in good health, free from symptoms of prostatic carcinoma. In a subsequent series of 38 patients treated by castration, stilbestrol, or both, the mortality has been 47%. Improvement by

endocrine therapy can be expected in 70% of cases, but it is only temporary.—V. F. M.

Bilateral Orchiectomy for Carcinoma of the Prostate Gland. Clinical Experience. EMMETT, J. L., and GREENE, L. F. [Mayo Clin., Rochester, Minn.] *J. A. M. A.*, **127**:63-67. 1945.

The present practice of the authors is to advise orchidectomy primarily for the relief of symptoms due to metastases. When orchidectomy is performed in conjunction with transurethral resection, the frequency of recurrent obstructive symptoms requiring subsequent prostatic resection is reduced. A group of patients without metastases is being treated with estrogens after transurethral resection in order to see if the course of the disease is appreciatively altered and whether preliminary estrogenic therapy will tend to nullify the palliative effects of castration should that become necessary later for control of symptoms. Data from 220 cases treated by orchidectomy are analyzed.—M. E. H.

Experience with Orchiectomy for Carcinoma of the Prostate. BUMPUS, H. C., JR., MASSEY, B. D., and NATION, E. F. [Pasadena, Calif.] *J. A. M. A.*, **127**:67-68. 1945.

Of 25 patients with prostatic carcinoma upon whom orchidectomy was performed 24 enjoyed temporary relief of symptoms. Forty per cent of the 15 patients observed for a year or more after operation have had a recurrence of symptoms. It is suggested that treatment with estrogens or by orchidectomy be delayed until symptoms of an advanced malignant condition are manifest and that estrogens and orchidectomy then be used separately and in succession rather than in conjunction.—M. E. H.

Use of Stilbestrol in Carcinoma of the Prostate. DONAHUE, C. D. [Eugene, Ore.] *Northwest Med.*, **43**:284-287. 1944.

A discussion of the relative merits of orchidectomy and medication by stilbestrol. Nineteen cases in which stilbestrol was used are described. There was improvement in all patients except in one, who did not tolerate the drug.—E. E. S.

The Effects of Biochemical Therapeusis in Carcinoma of the Prostate. Further Observations. HERBST, W. P. [Gallinger Hosp., Washington, D. C.] *J. A. M. A.*, **127**:57-59. 1945.

Sixteen case reports. The chemical treatment of carcinoma of the prostate (by estradiol dipropionate, diethylstilbestrol, ethinyl-estradiol, and pellets of estradiol benzoate) should not be considered as a cure. It offers a relatively simple method for the relief of pain and varying periods of satisfactory inhibition of extensive malignant processes. The observations already made should stimulate further constructive experimental and clinical efforts in this field.—M. E. H.

The Treatment of Skeletal Metastases Secondary to Carcinoma of the Prostate. PECK, R. I. [Univ. of Pennsylvania Med. Sch., Philadelphia, Pa.] *J. A. M. A.*, **127**: 17-19. 1945.

The author points out that orchidectomy or treatment with estrogens has not been advocated as a cure of carcinoma of the prostate with metastases but as a physiologic method for the relief of pain, and that this benefit may last 1 or more years. He cites 2 cases in which bilateral orchidectomy restored men with prostatic cancer and bone metastases to apparently good health, and led to considerable healing of the bone defects.—M. E. H.

Breast Changes Due to Diethylstilbestrol During Treatment of Cancer of the Prostate Gland. MOORE, G. F., WATTENBERG, C. A., and ROSE, D. K. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. A. M. A.*, **127**:60-62. 1945.

During treatment, the breast grossly becomes enlarged and often painful. There is proliferation of the duct epithelium with elongation and budding of the ducts themselves, increase in connective tissue stroma, increased vascularity throughout the connective tissue, and edema. Interstitial fibrosis and deposition of fat take place at a later stage. In the prostatic cancer tissue, after estrogen therapy, the cytoplasm is no longer swollen or foamy, and the nuclei become deep staining, pyknotic, and without orderly arrangement. As yet, no malignant changes in the breast have been found to arise from diethylstilbestrol therapy. Ten photomicrographs are presented.—M. E. H.

Interstitial Cell Tumors of the Testes. Report of Three New Cases. NATION, E. F., EDMONDSON, H. A., and HAMMACK, R. W. [St. Luke Hosp., Pasadena, and Los Angeles Co. Gen. Hosp., and Sch. of Med., Univ. of South. California, Los Angeles, Calif.] *Arch. Surg.*, **48**:415-422. 1944.

To 23 previously reported "acceptable" interstitial cell tumors of the testis, 3 new ones are added. One was malignant and occurred in an 82 year old man. The other patients were 30 and 34 years old. The tumors were characteristically nodular and yellow or yellow-brown; they were composed of cells similar to those of the hepatic parenchyma with rather small, round nuclei and many nucleoli.—W. A. B.

URINARY SYSTEM—MALE AND FEMALE

Renal Hemangioma: An Obscure Cause of Hematuria. ROTTINO, A., and MOHAN, H. [St. Vincent's Hosp., New York, N. Y.] *J. Urol.*, **51**:601-605. 1944.

Renal hemangioma is comparatively rare, approximately 50 cases having been recorded in the past 100 years. Three additional cases are presented with illustrations.—V. F. M.

Supernumerary Kidney with Clear Cell Carcinoma. EXLEY, M., and HOTCHKISS, W. S. [U. S. Marine Hosp., Norfolk, Va.] *J. Urol.*, **51**:569-578. 1944.

There are only 46 reported instances of supernumerary kidney. The authors report the first unquestionably authentic case of a malignant neoplasm in the anomalous organ.—V. F. M.

Tumors of the Renal Pelvis. MELEN, D. R. [Rochester, N. Y.] *J. Urol.*, **51**:386-394. 1944.

Two cases of renal pelvic neoplasm (1 benign and 1 malignant) are described, with illustrations.—V. F. M.

Leiomyoma of the Kidney Associated with Hemorrhagic Cyst. CRABTREE, E. G. [Boston, Mass.] *J. Urol.*, **52**:480-488. 1944.

A report of 2 cases of malignant leiomyoma of the kidney with a general discussion of this lesion.—V. F. M.

Primary Neoplasms of the Ureters: Report of Six Cases. Barnes, R. W., and Kawaichi, G. K. [Coll. of Med. Evangelists, Los Angeles, Calif.] *Urol. & Cutan. Rev.*, **48**:430-436. 1944.

Six instances of primary ureteral tumor are described, with roentgenograms. Ureteronephrectomy is the treatment of choice.—V. F. M.

Primary Epithelioma of the Ureter: A Follow-Up Study of Eighteen Cases with the Addition of Nine New Cases. Counseller, V. S., Cook, E. N., and Seefeld, P. H. [Mayo Clin., Rochester, Minn.] *J. Urol.*, **51**:606-615. 1944.

This disease is more frequent in men, more frequent on the right side, more commonly in the lower ureteral segment, and usually appears in persons over 50. Radical nephro-ureterectomy including a cuff of bladder is the treatment of choice. Follow-up data and illustrations are included.—V. F. M.

Primary Carcinoma of Ureter Treated by Excision and Anastomosis of the Cut Ends. McClelland, J. C. [Univ. of Toronto, Toronto, Canada] *J. Urol.*, **52**:522-525. 1944.

A case report.—V. F. M.

Certain Aspects of Primary Carcinoma of the Urinary Bladder. Saphir, O. [Michael Reese Hosp., Chicago, Ill.] *Urol. & Cutan. Rev.*, **48**:552-554. 1944; and *Proc. Inst. Med. Chicago*, **15**:281. 1945.

In 51 autopsies of patients with primary bladder carcinoma, distant metastases were found 16 times and local metastases, 5 times. The most common cause of death was pyelonephritis. Thirteen of the 16 patients with distant metastases had also invasion of the prostate or parametrium. Grading seemed of less prognostic value than determination of such invasion. The author poses the question whether a tumor primary in organ A and invading organ B will metastasize in the way that primary tumors of organ B do. Certain cases here presented suggest this may be true.—V. F. M.

Papillary Carcinoma of the Bladder with Extensive Metastases: Case-Report; Autopsy. Rathbun, N. P. [Brooklyn, N. Y.] *J. Urol.*, **51**:623-629. 1944.

An illustrated case report.—V. F. M.

Rhabdomyosarcoma of the Urinary Bladder. A Clinico-Pathological Case Report with a Review of the Literature, Including a Tabulation of Rhabdomyosarcoma of Prostate. Khoury, E. N., and Speer, F. D. [New York Med. Coll., and Flower and Fifth Avenue Hosp., New York, N. Y.] *J. Urol.*, **51**:505-516. 1944.

The authors describe the ninth proved case of this bladder condition. The first signs appeared 3 days after birth of the patient. The treatment was total cystectomy. At autopsy 11 months later residual tumor was found. The histogenesis and microscopic morphology are discussed.—V. F. M.

Extramural Rhabdomyosarcoma of the Neck of the Urinary Bladder. Hirsch, E. F., and Gasser, G. W. [St. Luke's Hosp., Chicago, Ill.] *J. Urol.*, **51**:517-519. 1944.

A male, aged 5, had a large rhabdomyosarcoma arising beneath the trigone and prostate. The authors consider this tumor might well be classed with those arising along the vas deferens rather than with those of prostatic origin.—V. F. M.

Oral Cavity and Upper Respiratory Tract

Mixed Tumor of the Soft Palate. Gidoll, S. H. [San Francisco, Calif.] *Arch. Otolaryng.*, **38**:166-168. 1943.

Report of a case of a simple, benign mixed tumor originating in parotid glandular tissue of the palate, and decreasing in size under radium therapy.—W. A. B.

Osteoma of the Frontal Sinus. Johnston, W. H. [Santa Barbara, Calif.] *Arch. Otolaryng.*, **38**:318-323. 1943.

Review of the literature and report of a case.—W. A. B.

Primary Carcinoma of the Left Sphenoid Cavity. Hardie, G. C., and Ahronheim, J. H. [Jackson, Mich.] *Arch. Otolaryng.*, **38**:497-501. 1943.

Report of case.—W. A. B.

Tumors of the Nose and Throat. New, G. B., and Foss, E. L. [Mayo Clin., Rochester, Minn.] *Arch. Otolaryng.*, **38**:505-519. 1943.

Report of recent progress in diagnosis and treatment, as presented in the literature, with 59 references.—W. A. B.

End Results of Treatment of Malignant Lesions of the Nasopharynx. New, G. B., and Stevenson, W. [Mayo Foundation, and Mayo Clin., Rochester, Minn.] *Arch. Otolaryng.*, **38**:205-209. 1943.

The study is based on 271 patients treated prior to 1937. Irradiation was the treatment of choice, by both radium introduced into the nasopharynx and radium or roentgen rays applied externally.

Squamous cell carcinoma occurred in 67.8% of the patients, various types of sarcoma in 15.9%, adenocarcinoma in 2.6%; in 13.7% there was no positive biopsy. Among those patients with carcinoma and without palpable lymph nodes, who were followed, 5 of 32 (15.6%) survived 5 years, and but 1 of 14 (7.1%) (operated upon earlier) survived 10 years. Among those with sarcoma without palpable lymph nodes, 8 of 16 (50%) survived 5 years, and 5 of 13 (38.5%) were alive after 10 years.—W. A. B.

Schmincke Tumor. Lymphoepithelioma of the Nasopharynx. Burman, H. J., and Burman, D. [Harlem Hosp., New York, N. Y.] *Arch. Otolaryng.*, **37**:835-844. 1943.

A review of the literature, with bibliography, and report of 3 cases.—W. A. B.

Carcinoma of the Larynx. LeJeune, F. E. [Tulane Univ. Sch. of Med., and Ochsner Clin., New Orleans, La.] *New Orleans M. & S. J.*, **97**:298-302. 1945.

A general clinical discussion. Stress is laid on the importance of investigating persistent hoarseness. The surgical technic of choice is suggested for various forms of the tumor.—E. E. S.

Sarcoma of the Larynx. Ferguson, G. B. [Durham, N. C.] *Arch. Otolaryng.*, **38**:265-269. 1943.

A report of 2 cases of fibrosarcoma. Although microscopically malignant, these tumors may follow a clinically benign course and are amenable to conservative surgical measures, even when the growth is large.—W. A. B.

Primary Carcinoma of the Trachea. Treatment with Intratracheal Radium; Radioactive Iodine Fails to Show Thyroid Origin. Pierson, P. H. [Stanford Univ. Sch. of Med., San Francisco, Calif.] *J. A. M. A.*, **126**:206-209. 1944.

A case report of an uncommon disease. As much as possible of the primary adenocarcinoma was removed

endoscopically; then intratracheal radium was applied. The patient is well 2 years after treatment. A test with radioactive iodine was indecisive in showing whether the tumor was of thyroid origin.—M. E. H.

Carcinoma of the Trachea. Ball, K. P. (for Young, R. A.) *Proc. Roy. Soc. Med.*, **38**:133-134. 1945.

Description of a case.—E. L. K.

Gastrointestinal Tract

Surgical Treatment and Clinical Manifestations of Benign Tumors of the Esophagus with Report of Seven Cases. Harrington, S. W., and Moersch, H. J. [Mayo Clin., Rochester, Minn.] *J. Thoracic Surg.*, **13**:394-414. 1944.

These tumors occur rarely. The majority are fibrous tumors arising in the submucosa, or are leiomyomas of the muscularis. Various means of removal are discussed. Reports of 7 cases are given; in 5, esophagotomy was performed, in 1, the tumor was removed orally by snare, and in the remaining instance, the esophagus was resected. Roentgenograms, photomicrographs, and diagrammatic drawings of operative findings are numerous and clear.—E. E. S.

Causes of Mortality Following Radical Resection of the Esophagus for Carcinoma. Garlock, J. H. [Mt. Sinai Hosp., New York, N. Y.] *J. Thoracic Surg.*, **13**:415-430. 1944.

This is a report of 60 operable cases of adenocarcinoma of the cardia and squamous carcinoma of the esophagus with a mortality of 48%. Six deaths were regarded as surgical failures. The majority of the additional deaths were attributed to cerebral accident or pneumonia.—E. E. S.

Palliative Treatment of Carcinoma of Esophagus: Report of a Case. Vinson, P. P. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **72**:24-25. 1945.

A case reported because of relief obtained by repeated dilation of the malignant stricture.—M. E. H.

Ulcerative Leiomyoma of the Stomach. Behrend, M. [Jewish Hosp., and Mt. Sinai Hosp., Philadelphia, Pa.] *J. Internat. Coll. Surgeons*, **7**:436-445. 1944.

A report of 2 cases and a review of the literature.—M. H. P.

Melanoma of the Small Intestine. Herbut, P. A., and Manges, W. E. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Arch. Path.*, **39**:22-27. 1945.

Reports of 5 cases, all metastatic, with reference to 25 cases from the literature. Intestinal obstruction and intussusception were frequent complications.—J. G. K.

Multiple Plasmocytoma of the Jejunum. Esposito, J. J., and Stout, A. P. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **53**:33-39. 1945.

This is a case report of a rare condition, illustrated with roentgenograms and photomicrographs.—E. H. Q.

Small Bowel Obstruction Due to an Unattached Secondary Tumour. Warren, R. F. [Toronto Gen. Hosp., Toronto, Canada] *Canad. M. A. J.*, **52**:44-47. 1945.

A case report. After the removal of a large fungating tumor of the jejunum and a small submucous one, the patient developed complete small bowel obstruction for the second time. A large, loose tumor within the ileum was removed. Presumably these growths were secondary adenocarcinomas from an undisclosed primary site.—M. E. H.

The Curability of Cancer of the Large Bowel. Heyd, C. G. [New York Post-Grad. Med. Sch., and Columbia Univ., New York, N. Y.] *Rev. Gastroenterol.*, **12**:23-30. 1945.

It is the responsibility of the medical profession to insist on the utilization of 4 simple procedures for the early diagnosis of cancer of the large bowel: (1) complete detailed history, (2) a digital examination, (3) the use of the sigmoidoscope, and (4) roentgenography with the aid of a barium enema before a gastrointestinal series is ordered. A wider utilization of exploratory laparotomy is urged. Annual examinations after the age of 50 would aid in the detection not only of cancers in the earliest stages but of benign epithelial tumors (adenomas and papillomas) that tend in varying degrees to undergo malignant changes.—M. E. H.

Two Stage Operation for Carcinoma of Transverse Colon Producing Duodenocolic Fistula. Linton, R. R. [Massachusetts Gen. Hosp., Boston, Mass.] *Arch. Surg.*, **48**:197-207. 1944.

Report of 2 cases in which right-sided colectomy and the Whipple type of operation designed for carcinoma of the pancreas were done.—W. A. B.

Ulcus Callosum Recti (Hochenegg). Mandel, F. [Hadassah Univ. Hosp., Jerusalem, Palestine] *J. Internat. Coll. Surgeons*, **7**:447-451. 1944.

Biopsy provides the only means for differentiating between ulcus callosum and carcinoma of the rectum, and even this is not infallible. Since it is now generally accepted that ulcus callosum recti is not a precancerous condition, radical operation is unnecessary. In the 2 cases reported here, temporary colostomy for a few months sufficed for healing of the ulcer.—M. H. P.

Liver

Role of Schistosomiasis in the Etiology of Cancer of the Liver in the Chinese. Hartz, P. H. [Pub. Health Service, Curaçao, Netherlands West Indies] *Arch. Path.*, **39**:1-2. 1945.

The author found 5 cases of liver cell cancer in 33 autopsies on Chinese men during 8 years in Curacao; in none of the 33 cases was there clinical, gross, or microscopic evidence of schistosomiasis.—J. G. K.

Lobectomy of the Liver. Report of Three Cases. Pickrell, K. L., and Clay, R. C. [Johns Hopkins Hosp. and Univ., Baltimore, Md.] *Arch. Surg.*, **48**:267-277. 1944.

Resection of the left lobe of the liver was performed in 3 cases: hemangioma, gumma, and carcinoma of the liver. All 3 patients survived the procedure. In the case of the gumma, a preoperative diagnosis of cancer was the reason for surgery.—W. A. B.

Spontaneous Cholecystoduodenal Fistula in a Patient with a Primary Hepatoma of the Liver. Pomeranz, R., Grady, H. G., Peelen, M., and Magnes, M. [Winter Gen. Hosp., Topeka, Kans.] *Radiology*, **43**:582-587. 1944.

A case report, with roentgenograms.—R. E. S.

THYROID

Lateral Aberrant Thyroid: Metastases to the Lymph Nodes from Primary Carcinoma of the Thyroid Gland. CLAY, R. C., and BLACKMAN, S., JR. [Johns Hopkins Univ., Baltimore, Md.] *Arch. Surg.*, **48**:223-228. 1944.

Report of 2 cases. The authors believe that the lesions known as lateral aberrant cervical thyroid should be interpreted as tumor metastases in cervical lymph nodes from small primary carcinomas in the homolateral lobe of the thyroid.—W. A. B.

MISCELLANEOUS

Tumor Seminar. STOUT, A. P., ET. AL [Columbia Univ. Coll. of Physicians and Surgeons, New York, N. Y.] *Texas State J. Med.*, **40**:366-391. 1944.

Instances of many different types of tumor are presented, with case histories, microscopic descriptions including photomicrographs of histological sections, and diagnoses.—J. L. M.

Carcinoma in Young Persons. MOREHEAD, R. P. [Bowman Gray Sch. of Med., Wake Forest Coll., Winston-Salem, N. C.] *Arch. Path.*, **38**:141-146. 1944.

A general discussion, and reports of: 3 cases of carcinoma of the body of the uterus arising in women 25, 29, and 27 years old; 2 cases of carcinoma of the cervix occurring in women aged 19 and 20; and 2 cases of carcinoma of the liver in boys aged 13 and 18.—J. G. K.

Analysis of the Relationship of Seminoma and Arrhenoblastoma to Teratoma. RHODEN, A. E. [Univ. of Arkansas Sch. of Med., Little Rock, Ark.] *Arch. Path.*, **37**: 251-252. 1944.

Discussion.—J. G. K.

Abdominal Silicosis (Due to Talcum Powder) and Cancer. SEELIG, M. G. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1162-1171. 1944.

Talcum powder granuloma, arising in the region subjected to surgery, complicated the postoperative course of 2 patients with cancer. Potassium bitartrate is recommended as a substitute for talc (which consists chiefly of magnesium silicate) in powdering rubber gloves used in surgery. Upon intraperitoneal administration to rats, talc and various other dusting powders caused granuloma, plaques, adhesions, or serous exudates, while potassium bitartrate did not.—J. L. M.

Metastatic Malignancy Treated by Radical Methods. LAWRENCE, E. A. [Yale Univ. Sch. of Med., New Haven, Conn.] *Connecticut M. J.*, **9**:23-24. 1945.

A report of 2 cases in which radical removal of adenocarcinoma metastases from the diaphragm and lung, respectively, apparently restored the patients to health. The primary tumors, in the cecum and uterus, had been removed before the metastases were observed. Usually, if multiple metastases are present, radical surgery is not warranted. Occasionally, however, sufficient palliation will be obtained by the removal of a single offending lesion to justify the time, expense, and danger of the procedure.—M. E. H.

Diagnosis and Treatment of Malignant Diseases in Veterans' Hospitals. BURTON, C. C. [Vet. Admin., Dayton, Ohio] *M. Bull. Vet. Admin.*, **21**:211-215. 1944.

The administrative organization for the handling of malignant neoplastic disease is outlined, and experience in 231 cases examined in 1943 by the Tumor Board of a single Veterans Administration Facility is summarized.—M. E. H.

The Results of Cancer Treatment. FARR, C. E. [New York, N. Y.] *New York State J. Med.*, **44**:1673-1674. 1944.

The author cites statistics from the literature to show the large number of 5 year cures of patients with cancer of various organs when such patients are treated early in the disease.—J. L. M.

Problems in the Postoperative Care of Cancer Patients. TREVES, N. [Memorial Hosp., New York, N. Y.] *New York State J. Med.*, **44**:2248-2254. 1944.

Advice on the care of the nonhospitalized patient.—J. L. M.

STATISTICS

Epidemiology of Peptic Ulcer. Vital Statistics. MORRIS, J. N., and TITMUSS, R. M. *Lancet*, **247**:841-845. 1944.

The data in this paper are derived from the returns of the Registrar-General for England and Wales. The death rate from cancer of the stomach in middle life has increased in recent years in men but not in women. Thus for the three periods 1911 to 1920, 1921 to 1930, and 1931 to 1935 the death rates per million at ages 45 to 55 were: for men 367, 413, 432, and for women 261, 259, 238. At ages 65 and onwards there has been an increase in both sexes. The Registrar-General's Decennial Supplements for 1921 and 1931 showed that the incidence of cancer of the stomach in males increased regularly with descent in the social scale from the richer to the poorer classes. The mortality from gastric ulcer shows a similar social relationship up to the age of 65; thereafter the relation is reversed, the incidence being heavier on the richer classes. Duodenal ulcer shows little relation to social class up to the age of 55; at later ages there is an increasing incidence upon the richer classes.—E. L. K.

The Incidence of Malignant Tumors in British West Indian and Panamanian Negro Autopsy Populations. TOMLINSON, W. J., and WILSON, L. A., JR. [Gorgas Hosp., Ancon, Canal Zone] *Cancer Research*, **5**:368-371. 1945.

A study was made of the malignant tumors in a British West Indian and a Panamanian Negro autopsy population in the Canal Zone. Because of unique circumstances these autopsy populations represent reliable cross sections of the findings in Negroes dying at all ages in this area. Malignant tumors occurred in 339 of 2,553 persons 10 or more years of age, or 13.3%. Among 1,839 males, malignant tumors occurred in 238, or 12.9%; among 714 females, malignant tumors occurred in 161, or 14.1%. Carcinoma was found 5 times as frequently as sarcoma and other malignant tumor types. The type of tumor, location, and age incidence are given in tabular form. The incidence of malignant tumors in this Negro autopsy population was essentially similar to that reported in Negro autopsy populations elsewhere and was substantially less than the incidence reported in white autopsy populations in the United States.—Authors' abstract.